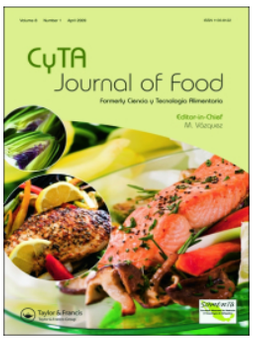

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Monitoring two different drying methods of Kakadu plum puree by combining infrared and chemometrics analysis

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ABSTRACT

The effect of two drying methods (oven and freeze drying) and the addition of maltodextrin to Kakadu plum puree samples (KP) (*Terminalia ferdianandiana*) were evaluated using mid (MIR) and near-infrared (NIR) spectroscopy. Dry powder samples were obtained using the oven and freeze-drying methods and seven levels of maltodextrin. Training (n = 32) and validation (n = 28) sets were developed for the prediction of moisture (M %), water activity (aw %), hydroxymethylfurfural (HMF) and vitamin C (VITC mg/100 g DM) based on NIR and MIR spectroscopy. Results from this study demonstrated the ability of spectroscopy combined with partial least squares (PLS) regression to monitor these parameters during drying. The standard error in cross validation (SECV) and the residual predictive deviation (RPD) values obtained were of 0.71% (RPD = 4.1) and 0.47% (RPD = 6.1) for M, 0.06% (RPD = 4.4) and 0.02% (RPD = 8.2) for aw, 0.73 (RPD = 3.3) and 0.72 (RPD = 3.3) for HMF, 465.7 mg 100 g DM (RPD = 3.0) and 289.3 mg 100 g DM (RPD = 4.8) for VITC, using MIR and NIR, respectively. The results from this study showed that MIR and NIR spectroscopies are capable of both measuring and monitoring the effect of drying and the addition of maltodextrin as a carrier to KP puree samples.

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NIR; MIR; vitamin C; hydroxymethyl furfural; Kakadu plum

PALABRAS CLAVE
NIR; MIR; vitamina C; hidroximetil furfural; ciruela de Kakadu

Monitoreo de dos métodos diferentes de secado del puré de ciruelas de Kakadu mediante la combinación de análisis de infrarrojos y quimiométricos

RESUMEN

Este estudio se propuso evaluar el efecto producido por dos métodos de secado (horno y liofilización) y por la adición de maltodextrina a muestras de puré de ciruela de Kakadu (KP) (*Terminalia ferdianandiana*) mediante espectroscopía de infrarrojo medio (MIR) e infrarrojo cercano (NIR). Con este objetivo se obtuvieron muestras de polvo seco utilizando los métodos de secado en horno y liofilización, además de adicionar siete niveles de maltodextrina. Así, se elaboraron conjuntos de práctica (n = 32) y validación (n = 28) para predecir la humedad (M %), la actividad del agua (aw %), el hidroximetil furfural (HMF) y la vitamina C (VITC mg/100 g MS) sobre la base de las espectroscopías NIR y MIR. Los resultados del estudio dieron cuenta de la capacidad de la espectroscopía, combinada con la regresión de mínimos cuadrados parciales (PLS), para monitorear estos parámetros durante el secado. El error estándar en la validación cruzada (SECV) y los valores de la desviación predictiva residual (RPD) obtenidos utilizando MIR y NIR fueron, respectivamente de: 0.71% (RPD = 4.1) y de 0.47% (RPD = 6.1) para M, 0.06% (RPD = 4.4) y 0.02% (RPD = 8.2) para aw, 0.73 (RPD = 3.3) y 0.72 (RPD = 3.3) para HMF, 465.7 mg 100 g de MS (RPD = 3.0) y 289.3 mg 100 g de MS (RPD = 4.8) para VITC. De esta manera, los resultados obtenidos permitieron constatar que las espectroscopías MIR y NIR son capaces de medir y de monitorear el efecto del secado y la adición de maltodextrina como portador de las muestras de puré de KP.

1. Introduction

The increasing market and consumer demands for high quality and healthy foods have created a need for efficient and accurate analytical methods for the quantification of bioactive compounds (e.g., antioxidants) in raw materials and finished products (Bunaciu et al., 2012; Cozzolino, 2015; García-Cañas et al., 2010; Ignat et al., 2001; Lu & Rasco, 2012; McGoverin et al., 2010). A large group of these bioactive compounds such as antioxidants and secondary metabolites can be found in plants and in many agricultural products having a wide range of biological activities and functions (Bunaciu et al., 2012; Cozzolino, 2015; García-Cañas et al., 2010; Ignat et al., 2001; Lu & Rasco, 2012; McGoverin et al., 2010).

Terminalia ferdinandiana is best known by its common name Kakadu plum (KP) (Brock, 2005; J.T. Gorman et al., 2019). This plant is one of the Australian native species that have the potential to significantly grow into commercial agribusiness (Brock, 2005; J.T. Gorman et al., 2019). The customary use of natural resources, including KP, by Australian Aboriginal people, spans over many thousand years (Brock, 2005; J. Gorman et al., 2016). This plant is utilised as food where the inner bark can be used for medicinal purposes (Brock, 2005; J. Gorman et al., 2016). The fruit of this plant has high levels of vitamin C and it is considered

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an excellent source of natural antioxidants such as phenolic compounds (e.g., gallic and ellagic acid; Brand et al., 1982; Konczak, 2009; Konczak et al., 2014, 2009; Netzel et al., 2007; Williams et al., 2014).

The KP fruit can be consumed directly, as a fresh fruit or kept under frozen conditions for further product development (Brand et al., 1982; Konczak, 2009; Konczak et al., 2014, 2009; Netzel et al., 2007; Williams et al., 2014). The food industry in Australia generally uses this raw material as an intermediate product, either as a puree or powder to be added as an ingredient in products such as beverages and muesli bars (Brand et al., 1982; Netzel et al., 2007; Williams et al., 2014). It is important that this intermediate KP products have consistent quality and retain bioactive compounds for use as a functional ingredient (Brand et al., 1982; Netzel et al., 2007; Williams et al., 2014). The loss of bioactive compounds is particularly modulated by the temperature and the moisture content during the drying processes (Brito de Sousa Lobato et al., 2018; Wu et al., 2020). Freeze drying or lyophilization is considered to be a dehydration method that is mild and can retain the bioactive compounds due to the use of low temperature (< 2°C) and vacuum conditions (Antal et al., 2011). In comparison to oven drying or hot-air drying, which uses hot air with constant flow and elevated temperature (>40°C) to remove moisture, this high temperature can lead to degradation of heat-sensitive compounds (Chua et al., 2019). Carrier agents such as starch and maltodextrin provide protection against oxidation as they form a protective layer around the molecules within the carrier matrix and provide stability during storage (Cai & Corke, 2000). Maltodextrin is a low-cost, odourless and bland compound which is ideal for the formulation of the carrier matrix and has demonstrated the retention of bioactive compounds during the drying process (Rodríguez-Hernández et al., 2005).

Rapid, low cost and reliable analytical methods will be of benefit in order to determine and quantify the nutritional and compositional value of the sample, as well as to evaluate the contribution to the overall bioavailability of different compounds and to allow the identification of specific biomarkers (Bunaciu et al., 2012; Cozzolino, 2015; García-Cañas et al., 2010; Ignat et al., 2001; Lu & Rasco, 2012; McGoverin et al., 2010). In recent years, both mid-infrared (MIR) and near-infrared (NIR) spectroscopy with its intrinsic benefits such as non-invasive, rapid, almost no necessary sample preparation, ability to perform on-/inline measurements, have been able to determine a wide range of physical and chemical parameters in a wide range of foods. These techniques have become widely used as analytical techniques in the so-called field of phyto-analytics and they have been included in pharmacopeia (Bunaciu et al., 2012; Cozzolino, 2015; García-Cañas et al., 2010; Ignat et al., 2001; Lu & Rasco, 2012; McGoverin et al., 2010). These methods provide a range of tools that can be used in a wide range of biological samples without the need for extraction, which can often lead to degradation of the antioxidant components (Bunaciu et al., 2012; Cozzolino, 2015; García-Cañas et al., 2010; Ignat et al., 2001; Lu & Rasco, 2012; McGoverin et al., 2010). These methods can also provide a high degree of precision when applied to the analysis of nutraceutical and antioxidant compounds as well as to monitor antioxidant activity in foods (Ignat et al., 2001; McGoverin et al., 2010; García-Cañas et al., 2010; Lu & Rasco, 2012; Bunaciu et al., 2012; Dong et al., 2013, 2014; Cozzolino, 2015). Other authors have also reported the use of vibrational spectroscopy (e.g., MIR and NIR) techniques to evaluate and monitor both the effect and the process of drying in different pharmaceutical ingredients and food matrices (Brito de Sousa Lobato et al., 2018; Pielesz, 2012; Watanabe et al. 2006; Wu et al., 2020; Zubak et al., 2020).

In this study, the effect of the drying method (oven and freeze drying) and the addition of maltodextrin as a carrier agent to Kakadu plum (KP) puree samples were evaluated using mid-infrared (MIR) and near-infrared (NIR) spectroscopy. Calibration models were developed using partial least squares regression to predict some of the routine parameters used to monitor the drying process.

2. Materials and methods

2.1. Samples

Commercial frozen Kakadu plum (KP) puree samples (ca. 15 Kg) were purchased from Traditional Homeland Enterprises Holding Co Pty Ltd (Morwell, Victoria, Australia), thawed at 4°C overnight and used for KP powder production. Maltodextrin (MALTO, food grade) with DE = 17–19 from Manildra Group (Gladesville, NSW, Australia) was completely pre-dissolved in water before adding to KP puree at different levels from 5 to 25% (w/w). The blend mixtures (approx. 500 g) were homogenized for 4 min at maximum speed using a high-speed homogeniser (Ultra-Turrax® T25, IKA, Germany) and the homogenous mixtures were smeared into stainless steel trays (50 x 30 cm) and subsequently subjected to either freeze dry (FZD) (Lab Gear SCANVAC, QLD, Australia) at −48°C for 7 days or conventional oven dry (OD) (Steridium, Brisbane, Australia) at 45°C for 3 to 4 days depending on the levels of added MALTO levels. The KP puree sample without MALTO was included as a control sample. After drying, the samples were ground into a fine powder using a Laboratory blender (Waring®8010/8011, NSW, Australia). Powder samples were sieved through a 200 µm sieve to obtain uniform particle size and stored in air-tight containers at −80°C for further analysis. All experiments were conducted in duplicate. A total of 60 samples were generated in this study (two drying methods and maltodextrin addition).

2.2. Reference methods

Water activity (aw) was determined by weighing approximately 2 g of the dry powder samples placed in a standard measuring cup for measurement of water activity using a LabTouch-aw water activity meter (Novasina AG, Labchen SZ, Switzerland) at a constant temperature of 25 ± 1°C and average stable scanning mode. The moisture content of the powder products was determined according to AOAC method 934.01 (AOAC, 2019). Approximately (3–4 g) of the dry powder samples were weighed into stainless steel dishes covered by lids and dried in a vacuum oven (Heraeus GmbH, Hanau, Germany) for approximately 16 h at 70°C under 250 mBar pressure to a constant weight where moisture content was expressed in percent. Extraction and analysis of 5-HMF were conducted followed the method previously published (Korbel et al., 2013), with modifications. Briefly, 200 mg KP powdered sample was

homogenized with 50% methanol (*v/v*) using a vortex. The homogenate was subsequently placed in an ultra-sonication bath for 30 min at room temperature, followed by centrifugation at 1800xg for 10 min (EppendorfCentrifuge5804, Hamburg-Eppendorf, Germany). Supernatants were retained, while residues were re-extracted twice followed the procedure described above. The supernatants were combined and subjected to UHPLC-PDA analysis employed a Waters UPLC-PDA system and a Waters HSS-T3 column (150 x 2.1 mm *i.d.*; 1.8 µm; 25 ^{0}C), with 95% aqueous acetonitrile containing 0.1% formic acid (v/v) as the mobile phase (0.3 mL/min) and isocratic elution. These compounds were identified and quantified at 285 nm based on an external calibration curve of HMF standard (HPLC grade) from Sigma-Aldrich (Castle Hill, NSW, Australia). HMF was expressed as mg/100 g DW. Extraction and analysis of vitamin C [Ascorbic acid (L-AA) and dehydroascorbic acid (DHAA)] in KP puree and KP powder products were conducted as previously reported by Phan et al. (2019) adapted from Campos et al. (2009). Briefly, 200 mg KP powder sample was extracted with 3% meta-phosphoric acid (*w/w*) containing 8% acetic acid (*v/v*) and 1 mM Ethylenediaminetetraacetic acid (EDTA). The reduction of dehydroascorbic acid (DHAA), which was also present in the extracts/samples, to L-AA was performed following the method of Spinola et al. (2012), prior to UPLC-PDA analysis. Total vitamin C (L-AA + DHAA) was determined using a Waters UPLC-PDA system and a Waters HSS-T3 column (150 x 2.1 mm *i.d*; 1.8 µm; 25 ^{0}C), with aqueous 0.1% formic acid as the mobile phase (0.3 mL/min) and isocratic elution. An aliquot of 2 µL of sample was injected into the UPLC system and the L-AA peak was detected at 245 nm, identified and quantified by comparison to a commercial standard (Williams et al., 2014). The LOD and LOQ for the method were 1.0 and 3.0 mg/L, respectively. An external calibration curve of L-AA was used for quantification and vitamin C was expressed as mg/100 g DW. The standard error for each of the reference methods was 0.003% for aw, 0.20% for moisture, 0.23 mg/100 g DW for HMF and 92.1 mg/100 g DW for Vitamin C.

2.3. Infrared measurements

The MIR spectra of dry samples was acquired using a Bruker Alpha spectrophotometer fitted with an attenuated total reflectance platinum diamond single reflection cell (Bruker Optics GmbH, Ettlingen, Germany). The MIR spectra were recorded using OPUS software version 8.5 (Bruker Optics GmbH, Ettlingen, Germany). Measurements were recorded in the spectral region, 4000 to 400 cm^{-1}. Each spectrum was computed using the average of 24 interferograms at a resolution of 4 cm^{-1}. Air was used as the reference background spectra and reset every 10 samples. The FT-NIR spectra of the dry powder samples were collected using a Bruker Tango-R spectrophotometer with a gold-coated integrating sphere (diffuse reflection). Samples were placed in a borosilicate-glass cuvette 10 mm diameter (Bruker Optics GmbH, Ettlingen, Germany). The reflectance spectra were recorded using OPUS software (version 8.5, Bruker Optics GmbH, Ettlingen, Germany) with 64 interferograms at a resolution of 4 cm^{-1} in the wavenumber range of 11,550 to 3950 cm^{-1}. Cuvettes were cleaned with 70% ethanol and dry with paper wipes between samples.

Table 1. Descriptive statistics for the parameters measured in the Kakadu dry powder samples.

Tabla 1. Estadísticas descriptivas de los parámetros medidos en las muestras de polvo seco de Kakadu.

	Mean	SD	Minimum	Maximum
M (%)	3.03	2.9	0.09	7.2
aw (%)	0.27	0.22	0.05	0.58
HMF (mg/100 g DW)	8.66	2.40	5.8	13.4
Vitamin C (mg/100 g DW)	19,041.4	1384	16,846	20,938

M: moisture, aw: water activity, HMF: hydroxymethylfurfural, DW: dry weight, SD: standard deviation.

M: humedad, aw: actividad del agua, HMF: hidroximetilfurfural, DW: peso seco, SD: derviación estándar.

2.4. Data analysis

The Unscrambler X software (v11, CAMO ASA, Oslo, Norway) was used for multivariate analysis. Both MIR and NIR spectra were pre-processed using the second derivative (Savitzky & Golay algorithm with a second polynomial order and a smoothing window size of 10 points; Savitzky & Golay, 1964). The second derivative was applied as it has been reported to be effective at correcting for baseline effects and slope of a spectrum (Savitzky & Golay, 1964). Principal component analysis (PCA) was performed to visualise the structure of the data, identify dominant features in the spectra and variation in the samples for further investigation (Bureau et al., 2019; Cozzolino et al., 2019; Naes et al., 2002). Calibration models between the spectra (MIR and NIR) and reference data were developed using partial least squares regression (PLS) using cross validation (Bureau et al., 2019; Naes et al., 2002). The optimal number of factors for the calibration model was selected based on the minimal value of the predicted residual sum of squares (PRESS) and the highest correlation coefficient (R^2) between actual and predicted values. The PLS models were evaluated in terms of the number of factors, standard error of cross-validation (SECV) and correlation coefficient. The residual predictive value (RPD) was used to evaluate the accuracy of the models (Bureau et al., 2019; Williams, 2001; P. Williams et al., 2017). Please note that the calibration models developed were only used to test the ability of spectroscopy to monitor the drying process.

3. Results and discussion

3.1. Spectra interpretation

The second derivative of the absorbance values originated from the dominant features in the MIR range is presented in Figure 1. It was observed that the main peaks were associated with the O-H regions mainly associated with the water content of the samples. These regions were more dominant in the OD samples compared with the FZD ones (Figure 1 panel A). In the MIR second derivative spectra, peaks were observed between 3500 and 3000 cm^{-1} predominantly associated with stretching vibrations of hydroxyl groups (O-H) of water (Bunaciu et al., 2012; Schoenbichler et al., 2014; Stuart, 1996). Peaks between 3000 and 2700 cm^{-1} might be also associated with asymmetric (2917 cm^{-1}) and symmetric (2859 cm^{-1}) vibration of C-H bonds of aliphatic CH_2 groups, mainly related to the presence of lipids (Bunaciu et al., 2012; Schoenbichler et al., 2014; Stuart, 1996). In addition, peaks

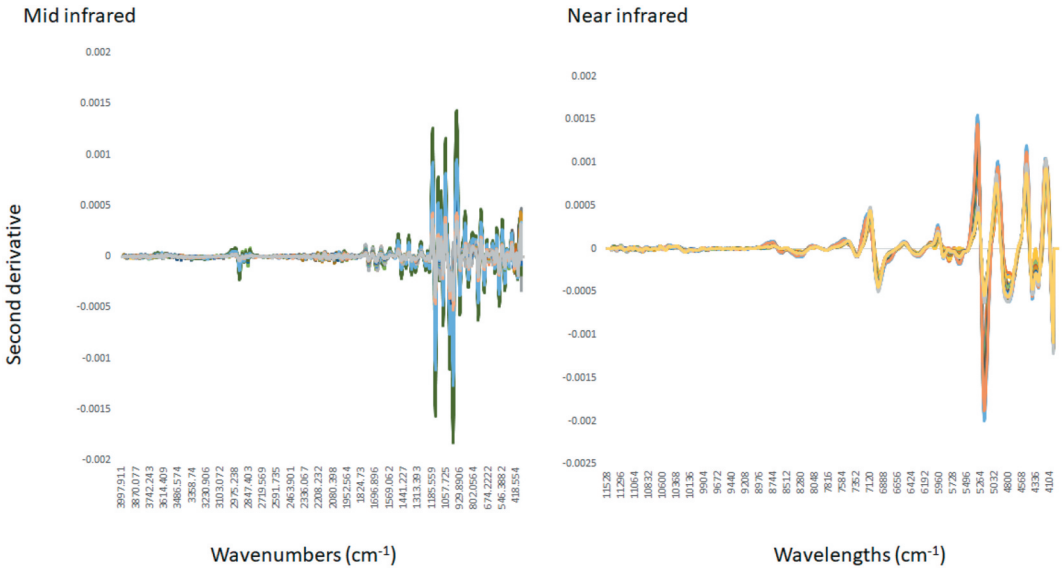

Figure 1. Second derivative of Kakadu plum dry powder samples analysed using either mid- or near-infrared spectroscopy.

Figura 1. Segundo derivado de las muestras de polvo seco de la ciruela de Kakadu analizadas mediante espectroscopía de infrarrojo medio o cercano.

related to both lipid structures and esters groups were identified at around 1730 cm^{-1} (Stuart, 1996). The region between 1694 cm^{-1} and 1441 cm^{-1} is mostly related to the amide groups corresponding to proteins as well as with the presence of polysaccharides in the KP powder fruit samples analysed (Bunaciu et al., 2012; Schoenbichler et al., 2014; Stuart, 1996). The amide I was assigned with the frequency around 1645 cm^{-1} (N-H bending) while the amide II was associated with 1542 cm^{-1} (Stuart, 1996). The region between 1240 and 800 cm^{-1} has been reported with the H-O-C stretch vibrations of the saccharide ring, polysaccharide molecules and cellulose structures (Bunaciu et al., 2012; Schoenbichler et al., 2014; Stuart, 1996). In addition, frequencies between 1040 and 800 cm^{-1} might be related to polysaccharides (e.g., starch) (Stuart, 1996).

The NIR second derivative spectra of the KP powder samples analysed showed specific bands around 8550 cm^{-1} (C–H, second overtone) around 5800 cm^{-1}, the C–H stretch first overtone CH_2 bond vibrations (C–H, stretch the first overtone) appearing at around 5680 cm^{-1} (Figure 1 panel B) (Schoenbichler et al., 2014; Workman & Weyer, 2012). Lipid structures might be also associated with absorbance around 4330 cm^{-1} (C–H bending the second overtone; Schoenbichler et al., 2014; Workman & Weyer, 2012). In addition, around 4855 cm^{-1}, protein vibrations are located due to the amide combination band of $CONH_2$ (Schoenbichler et al., 2014; Workman & Weyer, 2012). The broad absorbance band in a range of 7100–6100 cm^{-1} is associated with the N–H stretching (first overtone) of proteins and the absorbance associated with water content (O–H symmetric and asymmetric stretching combination, first overtone). Furthermore, a combination band of water appears at a wavenumber around 5155 cm^{-1} due to the O–H stretching and H–O–H bending combination tones (Schoenbichler et al., 2014; Workman & Weyer, 2012). In the NIR spectra, the OD samples showed higher absorbance values compared with the FZD.

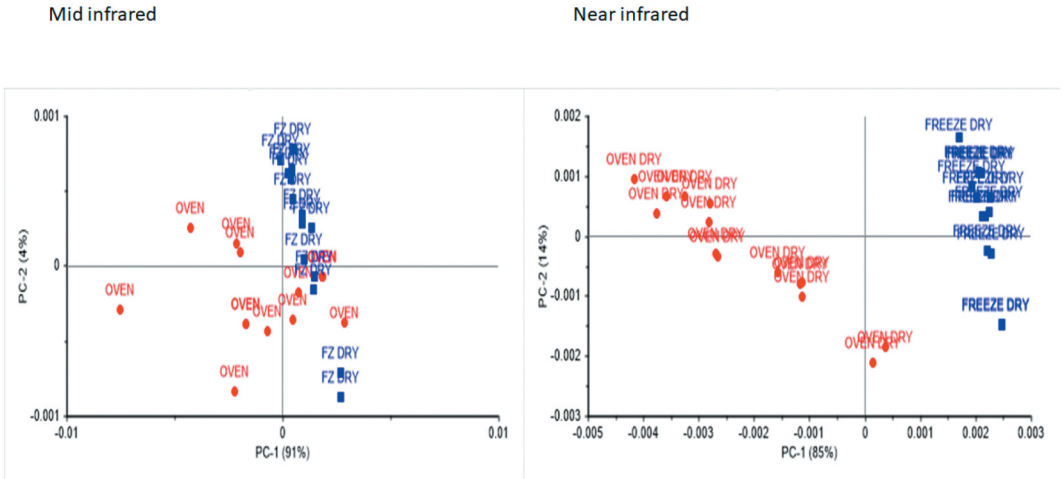

Figure 2. Principal component score plot for the analysis of Kakadu plum dry samples analysed using mid- and near-infrared spectroscopy.

Figura 2. Gráfico de puntuación del componente principal para el análisis de las muestras secas de ciruela de Kakadu analizadas mediante espectroscopía de infrarrojo medio y cercano.

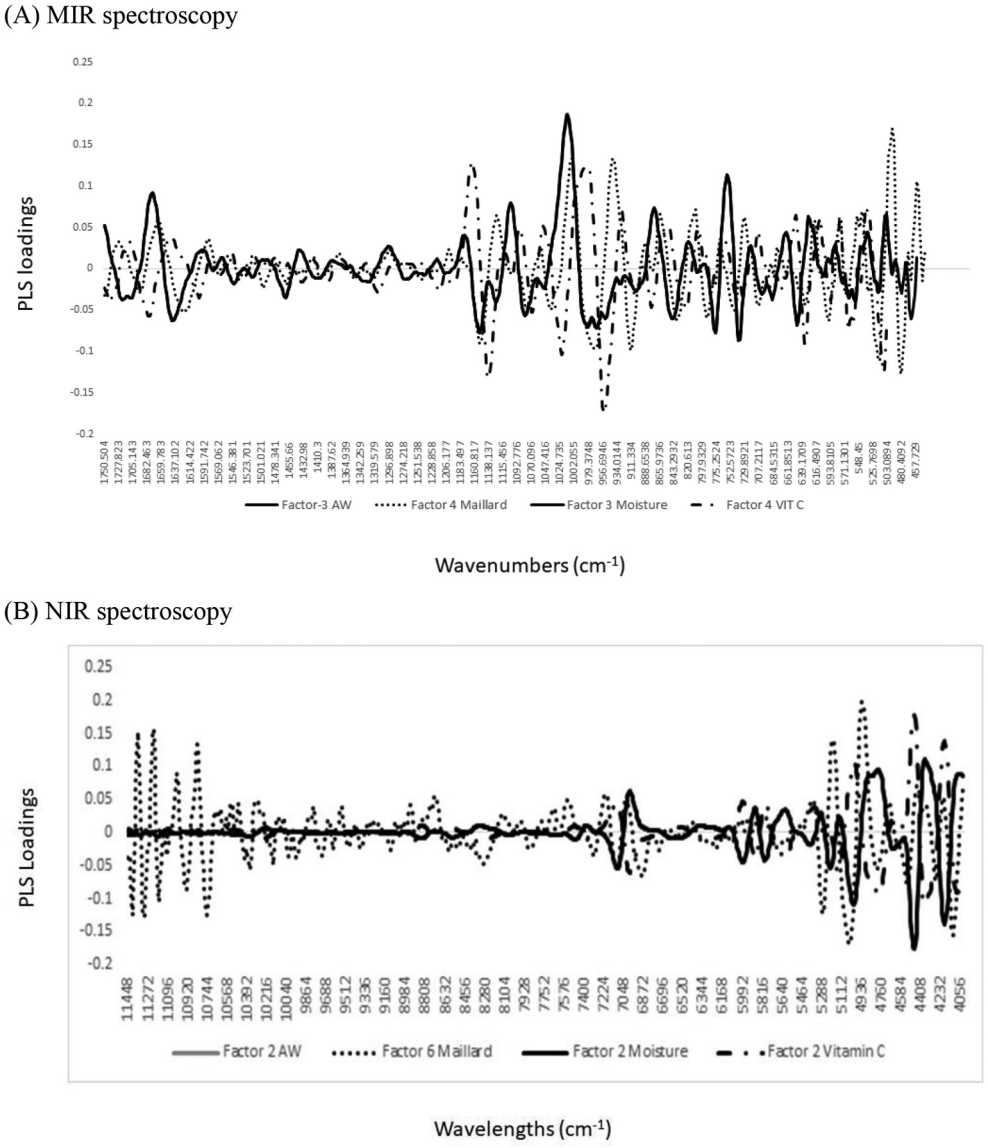

Figure 3. Loadings derived from the partial least squares regression models used to predict the chemical parameters in Kakadu pm dry samples using (A) mid- and (B) near-infrared spectroscopy.

Figura 3. Cargas derivadas de los modelos de regresión de mínimos cuadrados parciales utilizados para predecir los parámetros químicos en las muestras secas de ciruela de Kakadu utilizando (A) espectroscopía del infrarrojo medio y (B) del infrarrojo cercano. (A) Espectroscopía MIR. (B) Espectroscopía NIR.
(A) MIR spectroscopy. (B) NIR spectroscopy.

3.2. Principal component analysis

To determine the similarities and differences in the infrared spectra of the samples dried using the two drying methods a PCA analysis was applied. Figure 2 (Panel A) shows the scores plot of the KP powder samples analysed using both MIR and NIR spectroscopy. Using the MIR spectral data, the first two principal components explained 95% of the variability in the dataset. The samples were clustered based on the drying method along with the first principal component while PC2 explained the changes in the MIR spectra associated with the addition of maltodextrin to the powder samples. Figure 2 (Panel B) shows the scores plot for the KP powder samples analysed using NIR spectroscopy. Similarly, the first two principal components explained 99% of the variability in the data. A similar trend as the one obtained using the MIR data was observed for the samples analysed using NIR spectroscopy (e.g., PC1 was associated with water content while PC2 was associated with the addition of maltodextrin).

3.3. Partial least squares regression models

Table 1 shows the descriptive statistics (average, range, standard deviation) for the moisture, water activity, hydroxymethylfurfural and vitamin C measured in the KP powder samples used to develop the PLS calibration models. These results indicated a wide range of variation in the data set due to the different drying methods and levels of maltodextrin added to the KP puree samples. The variability in the data set was considered adequate to develop calibrations for this bioactive compound using either MIR or NIR spectroscopy.

Table 2 shows the cross-validation statistics for moisture, water activity, hydroxymethylfurfural and vitamin C analysed

Table 2. Cross-validation statistics obtained for the prediction of chemical parameters in Kakadu dry powder samples analysed using either mid- or near-infrared spectroscopy.

Tabla 2. Estadísticas de validación cruzada obtenidas para la predicción de los parámetros químicos en las muestras de polvo seco de ciruela de Kakadu analizadas mediante espectroscopía de infrarrojo medio o cercano.

	R^2	SECV	Bias	Slope	RPD	LV
MIR (n = 28)						
M (%)	0.95	0.71	0.03	0.96	4.1	3
aw (%)	0.92	0.06	0.003	0.97	4.4	5
HMF (mg/100 g DW)	0.90	0.73	−0.05	0.91	3.3	4
Vitamin C (mg/100 g DW)	0.88	465.7	25.9	0.88	3.0	4
NIR (n = 28)						
M (%)	0.97	0.47	0.007	0.97	6.1	2
aw (%)	0.98	0.02	0.0004	0.98	8.2	2
HMF (mg/100 g DW)	0.92	0.72	−0.06	0.87	3.3	6
Vitamin C (mg/100 g DW)	0.96	289.3	2.1	0.97	4.8	2

R^2: coefficient of determination; SECV: standard error of cross validation; RPD: residual predictive deviation; LV: latent variables; M: moisture; HMF: hydroxymethylfurfural; aw: water activity; n: number of samples.

R^2: coeficiente de determinación; SECV: error estándar de validación cruzada; RPD: desviación predictiva residual; LV: variables latentes; M: humedad; HMF: hidroximetilfurfural; aw: actividad del agua; n: número de muestras.

using both MIR and NIR spectroscopy. The standard error in cross validation (SECV) and the residual predictive deviation (RPD) values obtained were of 0.71% (RPD = 4.1) and 0.47% (RPD = 6.1) for M, 0.06% (RPD = 4.4) and 0.02% (RPD = 8.2) for aw, 0.73 (RPD = 3.3) and 0.72 (RPD = 3.3) for HMF, 465.7 mg 100 g DM (RPD = 3.0) and 289.3 mg 100 g DM (RPD = 4.8) for VITC, using MIR and NIR spectroscopy, respectively. The RPD values obtained for moisture, water activity and vitamin C measured in the KP powder samples were equal or higher than 4, indicating that these calibrations can be used for the quantitative determination of these parameters using either MIR or NIR spectroscopy (Bureau et al., 2019; Williams, 2001; P. Williams et al., 2017). However, a semi-quantitative (low, medium or high HMF) calibration model was obtained for the measurement of HMF using either MIR or NIR spectroscopy (RPD≥ 3) (Bureau et al., 2019; Williams, 2001; P. Williams et al., 2017). The low performance for the HMF calibrations might be related to the low SD for this parameter or the lack of information about compounds derived from the Maillard reaction contained in the infrared spectra.

The PLS loadings for the optimal calibration models developed for the measurement of the chemical parameters in KP powder samples are shown in Figure 3. Similar IR spectral regions as described in Figure 1 were used by the PLS algorithm during calibration development using either MIR or NIR spectroscopy. In both MIR and NIR calibration models, PLS loadings for moisture and water activity were almost identical, corresponding with frequencies or wavelengths associated with O-H groups (Bunaciu et al., 2012; Schoenbichler et al., 2014; Stuart, 1996; Workman & Weyer, 2012). The PLS loadings for the measurement of vitamin C and HMF were different from those observed for water.

4. Conclusions

The results from this study showed that MIR and NIR spectroscopies are capable of both measuring and monitoring the effect of drying and the addition of maltodextrin as a carrier to KP puree samples. The use of these methodologies offers considerable advantages compared with the use of routine methods of chemical analysis, as the sample preparation is simple. The practical implications of this study demonstrated that vibrational spectroscopy provides valuable benefits for the food industry allowing for the rapid monitoring of the drying process of a native food like Kakadu plum puree. The utilization of these techniques also offers the possibility to develop calibrations between spectra and the reference methods in order to measure several parameters simultaneously and therefore, reducing the time and cost of analysis. The utilization of rapid and low-cost tools for measuring quality can be implemented throughout the food value chain and they will provide with huge benefits to the natural food industry.

Acknowledgments

The authors acknowledge the Traditional Owners of the lands on which the *Terminalia ferdinandiana* fruits were harvested respecting the knowledge and experience the Traditional Owners hold regarding the care, harvest and use of these plants.

Disclosure statement

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Measurement of total soluble solids and moisture in puree and dry powder of Kakadu plum (*Terminalia ferdinandiana*) samples using hand-held near infrared spectroscopy

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Abstract

Recent research has shown the potential of portable and handheld NIR instruments to monitor and measure the composition of fruits and vegetables. Current research has also shown the possibility of using portable instruments as tools to monitor composition along the entire food value chain. The objective of this study was to evaluate two sample presentation methods (dry powder and fruit puree) to measure total soluble solids (TSS) and moisture (M) in wild harvested Kakadu plum (KP) (*Terminalia ferdinandiana,* Combretaceae). Kakadu plum is an endemic plant of Australia that contains high concentrations of vitamin C, ellagic acid as well as other bioactive compounds. These properties make this plant of high economic and social importance for the Aboriginal communities of Australia. Fruit samples were wild harvested in January 2020 from locations in the Kimberley region (Western Australia, Australia) and analysed using both reference and NIR spectroscopic methods. The SECV and RPD values in cross validation were 0.65% (RPD: 2.2) and 0.22% (RPD: 4.2) to predict M and TSS in the KP dry powder samples. The SECV and RPD values obtained in cross validation for the KP fruit puree samples were 0.56% (RPD: 2.8) and 0.24% (RPD: 3.8) for M and TSS, respectively. The results of this study demonstrated the ability of NIR spectroscopy to measure M and TSS in wild harvest fruit. These findings can be also utilised by the Aboriginal communities to develop a grading/sorting system to rapidly screen and evaluate relevant chemical parameters associated with fruit quality and safety.

Keywords

NIR, Kakadu plum, total soluble solids, moisture, linear variable filter

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Introduction

Native or indigenous plants species have found application in the functional foods, nutraceuticals, and cosmetic industries as raw materials or ingredients.[1–7] The Australian native food industry has expanded following the increase in research and development in phytochemical compounds as consequence of their importance in providing health benefits.[1–7] Kakadu plum (KP) (*Terminalia ferdinandiana*, Excell. Combretaceae) is an endemic plant of economic importance in Australia.[1–9] This fruit tree is a semi-deciduous plant found in the Northern Territory and Western Australia (Kimberley region).[1–9] Fruit of KP can be consumed fresh or as powders,[1–9] while the bark can be used to produce a wide range of products with medicinal properties.[1–9] The increasing demand for functional foods and nutraceuticals has increased the need to provide information on the safety and quality of using these native plant materials as ingredients in a wide range of food products.[10] Monitoring the composition of these plant materials is of importance in order to fulfil safety requirements and to assure the quality of these products. Several analytical methods are currently implemented and used in the laboratory

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during the routine analysis of these bio active compounds (e.g. HPLC). However, the use of conventional analytical methods in remote regions for most of the time is not practical. Thus, the utilization of rapid analytical methods based on near infrared (NIR) spectroscopy might be an option to monitor the chemical composition of native plant species.

Recent research has showed the potential of portable and handheld NIR instruments to monitor and measure the composition of fruits and vegetables.[11,12] Where the prospect of using portable instruments is proving to be promising as tools to monitor composition in a wide range of agricultural products. These findings are leading to a grow on the number applications of NIR spectroscopy in the field (e.g. on farm analysis).[11,12] Most of the current research and applications of NIR spectroscopy on fruits, native plants and/or agricultural products are based on the use of conventional harvested fruit, however, not many reports were found on the use of NIR spectroscopy to either measure or monitor the composition of wild harvested fruit.[11–13] Australian Aboriginal communities wild harvest KP fruit, therefore, it is important to monitor their composition in order to make harvesting a sustainable practice as well as target high quality fruit.[7–10] The development of rapid analytical methods will help to define optimal harvesting times as this can contribute to monitor the composition of these plant products in remote communities. As wild harvest practices require time consuming postharvest processing, the development of an objective method of analysis will allow for a better screening of samples in order to select fruit to be processed and/or stored. Overall, the development of objective and rapid methods will help Aboriginal communities in Australia to better schedule harvesting time and to provide with more accurate tools to pre-screen fruit samples before further processing.

It is well known that sample presentation can influence the NIR spectra and thus the results obtained after the use of chemometric techniques (e.g. classification, regression or calibration development).[14,15] The objective of this study was to evaluate two sample presentation methods (dry powder and fruit puree) to the instrument to measure total soluble solids and moisture in wild harvested Kakadu plum samples.

Materials and methods

Previously characterised fruit samples of Kakadu plum (*Terminalia ferdinandiana,* Combretaceae) were wild harvested in January 2020 at two locations in the Kimberley region (Western Australia, Australia). The two locations were selected as they have distinctive environmental and climatic characteristics (e.g. rainfall, temperature). In this study, ten KP trees from each site or location were randomly selected where approximately 50-100 fresh fruits were collected from each tree. The samples were transported under refrigerated storage in plastic bags, stored frozen at −80°C and thawed at room temperature (20°C) before analysis. Fruit samples from each tree were blended into a puree using a mortar and pestle. After acquiring the spectral data, the puree samples from each tree were then freeze-dried under vacuum (Lindner & May Ltd, Windsor, Brisbane), finely ground in a Retsch MM301 cryomill (Retsch GmbH, Haan, Germany) to provide a uniform powder and stored at −80°C (48 hrs) before reference analysis.

Total soluble solids (TSS, °Brix) were determined using a digital refractometer (Hanna Instruments Ltd., Leighton Buzzard, UK).[16,17] Moisture (M %) in the sample was determined by the method described in William and collaborators.[16,17] Triplicate puree samples (3–4 g) were weighed into stainless steel dishes covered by lids and dried for approximately 16 h at 70°C under 250 mBar pressure in a vacuum oven (Heraeus GmbH, Hanau, Germany) to a constant weight and moisture is expressed in per cent.[16,17] The standard error of the laboratory (SEL) method for the measurement of TSS and M is 0.31% and 0.19%, respectively.

The NIR spectra of the KP fruit puree (n = 60) samples were acquired first, and then the same sample analysed as freeze dry powder using a portable NIR spectrometer (MicroNIR 1700, Viavi Solutions, Milpitas, CA, USA) operating in the 950–1600 nm wavelength range, with a spectral resolution of 10 nm with no moving parts. The NIR instrument, a linear variable filter (LVF) spectrometer was connected through an USB interface to a notebook computer running proprietary software (MicroNIR Pro v3.1, Viavi Solutions, Milpitas, CA, USA) for the acquisition of diffuse reflectance spectra of the samples.[18] The controlling parameters for spectral data acquisition were set at 50 ms integration time and averaging of 50 scans. The reference spectra for absorbance/reflectance calculation was collected using Spectralon®. For each dry powder sample, an average spectrum was obtained from four scans from the container.[18]

Principal component analysis (PCA) and partial least squares (PLS) regression were used to interpret the data and to develop calibrations for M and TSS.[19,20] The Unscrambler software (version 11; CAMO Analytics, Oslo, Norway) was used to pre-process the data and develop the models. The spectra of both powder and puree samples were pre-processed using Savitzky–Golay second derivative (second polynomial order, 10 smoothing data points).[21] Samples were divided into calibration (n = 40) and validation (n = 20) sets using the Kennard-Stone algorithm available in the Unscrambler software.[22] The PLS calibration models were developed for total soluble solids (%) and moisture (%) using cross validation (leave one out).[19,20,23]

Results and discussion

Figure 1 shows the raw (Panel A) and second derivative (Panel B) NIR spectra of KP samples scanned as fruit puree or dry powder. The second derivative NIR spectra of the KP fruit puree samples showed three main wavelengths around 994 nm, 1174 nm and 1428 nm.[24] The wavelength at 1428 nm might be associated with either OH stretch first overtone or N-H related with water and protein content,[24] respectively. Wavelengths at 994 nm and 1180 nm are associated to CH stretch overtones (third and second overtone) mainly related with carbohydrates and other organic compounds present in the KP samples analysed.[24] The second derivative of the NIR spectra of the dry powder samples showed the contribution of wavelengths around 1031 nm, 1199 nm, 1292 nm, 1433 and 1459 nm.[24] Wavelengths at 1428 nm (OH stretch first overtone) and 1459 nm (N-H amine group) are mainly associated with water, protein and phenolic compounds.[24] The wavelength at 1199 nm is associated to CH stretch overtones (third and second overtone), mainly related with carbohydrates and other organic compounds present in the KP samples analysed.[24]

A PCA analysis of both KP samples analysed as dry powder and puree using NIR spectroscopy showed a separation between samples according to location (data not shown). Differences in TSS content (approx. 20%) and M (approx. 5%) were also observed between the KP samples sourced from the two locations.

Table 1 shows the cross validation and validation statistics for M (%) and TSS (%) for samples analysed as dry powder and fruit puree. The standard

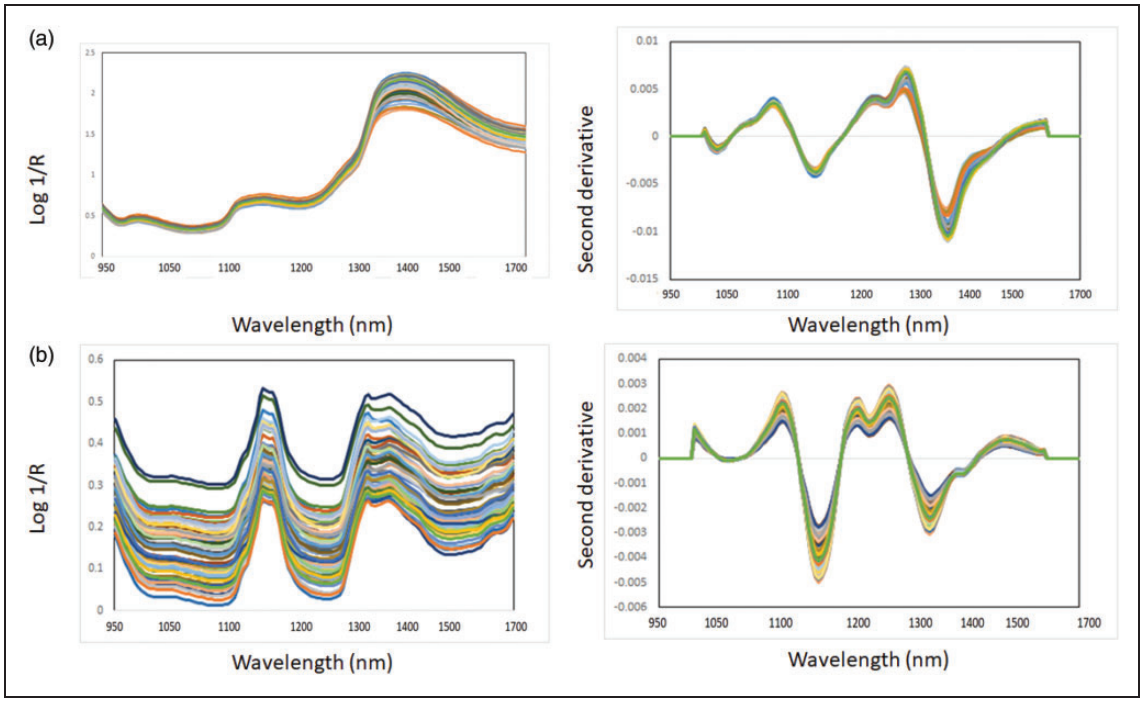

Figure 1. Raw and second derivative NIR spectra of Kakadu plum puree (A) and dry powder (B) samples analysed using near infrared reflectance spectroscopy.

Table 1. Cross validation and validation statistics for the measurement of moisture and total soluble solids in Kakadu plum dry powder and puree samples analysed using near infrared reflectance spectroscopy.

		Calibration (n = 40)						Validation (n = 20)	
		R^2	SECV	Slope	Bias	LV	r^2	SEP	RPD
Powder	M (%)	0.75	0.65	0.83	0.02	7	0.71	0.59	2.2
	TSS (°Brix)	0.79	0.22	0.80	0.002	7	0.70	0.20	4.2
Puree	M (%)	0.90	0.53	0.86	0.06	13	0.86	0.68	2.8
	TSS (°Brix)	0.86	0.24	0.85	0.001	6	0.72	0.58	3.8

R^2: coefficient of determination; SECV: standard error of cross validation; SEP: standard error of prediction; RPD: SD/SECV; LV: latent variables; M: moisture; TSS: total soluble solids, cv: cross validation; r^2: coefficient of determination in validation.

error of cross validation (SECV) and the residual predictive deviation (RPD = SD/SECV) in cross validation were used to evaluate the predictive ability of the PLS models developed.[19,20,23] The SECV and RPD values in cross validation obtained for the prediction of M and TSS in the KP dry powder samples were 0.65% (RPD: 2.2) and 0.22% (RPD: 4.2), respectively. The standard error of prediction (SEP) obtained for the prediction of M and TSS in the validation set of KP dry powder samples was 0.59% and 0.2%, respectively. The SECV and RPD values in cross validation obtained for the prediction of M and TSS in the KP fruit puree samples were 0.56% (RPD: 2.8) and 0.24% (RPD: 3.8), respectively. The SEP obtained in the validation set (KP fruit puree samples) was 0.68% and 0.58% for M and TSS, respectively. The PLS calibration models developed using dry powder samples explained between 75% and 78% of the variation related with M and TSS while 85% to 86% of the variation was explained using the KP fruit puree samples. The observed differences in the PLS models might be associated with the sample presentation (dry powder vs fruit puree) as well as with losses in moisture during the freeze-dry process used to obtain the powder samples. Figure 2 shows the scatter plot of the reference versus the NIR predicted values using the validation set (n = 20). Three and two outliers were observed for the prediction of TSS in the KP fruit puree and dry powder samples, respectively. No outlier samples were observed for the prediction of M.

The regression coefficients were analysed and interpreted for each of the models developed (Figure 3). The relationship between the wavelength and PLS latent variables or coefficients of regression imply these wavelengths contribute in explaining the models developed.[24–26] The absolute value of the PLS regression coefficient indicated the contribution of that individual wavelength to the model.[24,25] For example, it has been reported that when PLS models are developed for the same parameter using different scanning positions they can use different wavelengths or coefficients of regression. The coefficients of regression used by the PLS calibrations models for the measurement of M in the KP dry powder samples were evaluated. The main coefficients were observed around 1044 nm (C-H combination), 1149 nm (C-H and C = O), 1248 nm (C-H), 1335 nm (first overtone of C-H combination) and 1435 nm (O-H and N-H).[24] The coefficients for the prediction of M in the KP fruit puree samples, were observed at 1236 nm (C-H), 1310 nm (first overtone of C-H combination), 1372 nm (C-H), 1420 nm (O-H), 1477 nm (O-H) and 1546 nm (O-H).[24–26] The most important coefficients of regression used to develop the TSS calibration models using the KP dry powder samples were observed at 1106 nm (C-H), 1199 nm (C-H), 1316 nm (C-H), 1428 nm (N-H) and 1502 nm (N-H) while only three 1143 nm (C-H), 1202 nm (C-H) and 1527 nm (C-H and N-H) were observed when KP fruit puree samples were analsyed.[24–26] In this study, it was observed that the

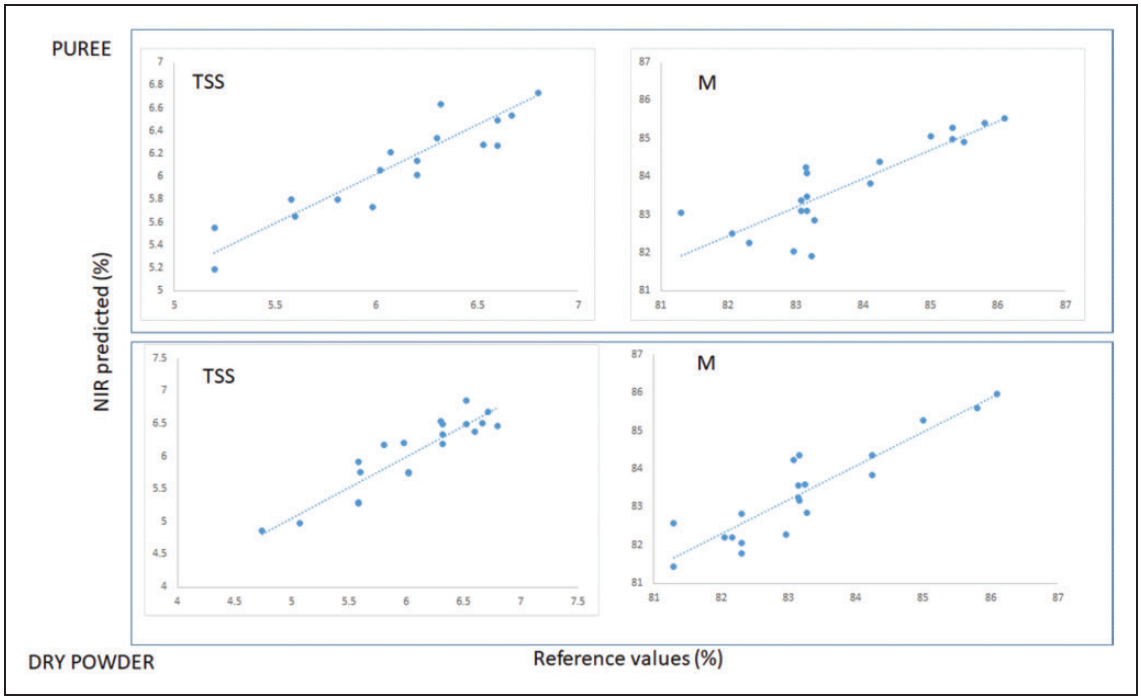

Figure 2. Scatter plot of the reference versus the NIR predicted values for the measurement of moisture and total soluble solids in the fruit puree and dry powder Kakadu samples analysed using near infrared reflectance spectroscopy.

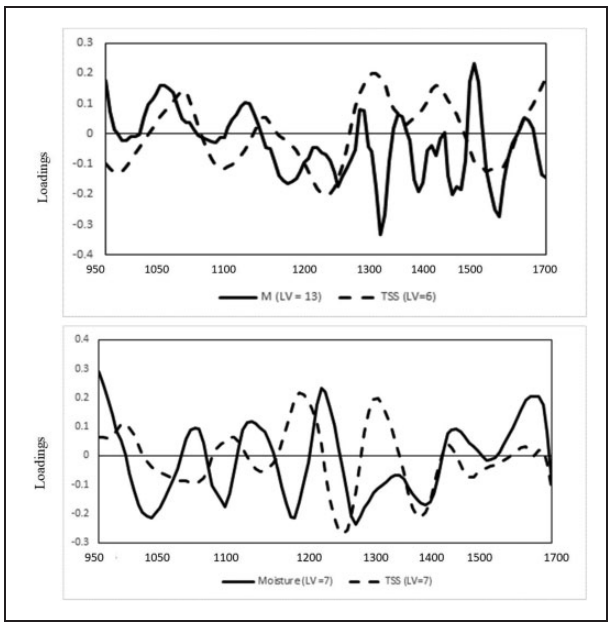

Figure 3. Coefficients of regression derived from the PLS models used to measure moisture and total soluble solids in the set of dry powder and fruit puree Kakadu samples using near infrared reflectance spectroscopy.

Picture of Kakadu plum (*Terminalia ferdinandiana* Excell.) (Source: Eshetu Bobasa and Yasmina Sultanbawa).

PLS calibration models for the same parameter utilised similar wavelengths and these might indicated that the sample presentation (dry powder vs fruit puree) might not have a greater effect on the information collected by the NIR instrument.

Conclusions

The results of this study showed the potential of a hand-held NIR spectrometer to predict M and TSS in wild harvest KP plum samples (fruit puree and dry powder). The results of this study will be of great value in order to develop protocols to schedule harvest in wild fruit KP plums. These findings can be also utilised by the Aboriginal communities to develop a grading/sorting system to rapidly screen and evaluate relevant chemical parameters associated with fruit quality and safety.

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Declaration of conflicting interests

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Assessing the interaction between drying and addition of maltodextrin to Kakadu plum powder samples by two dimensional and near infrared spectroscopy

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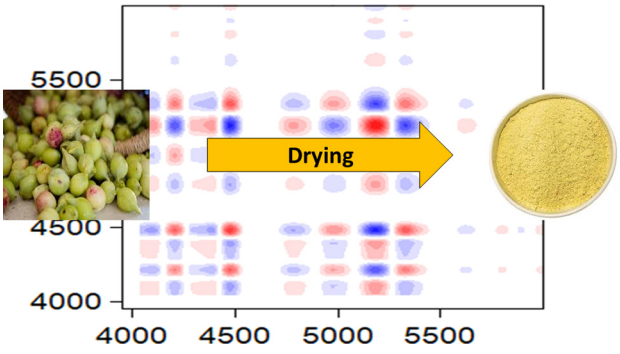
HIGHLIGHTS

- NIR spectroscopy was evaluated to monitor the drying process of Kakadu plum.
- 2 dimensional spectroscopy was utilised to interpret the NIR spectra.
- Freeze dried samples had similar moisture content compared with oven dry.

GRAPHICAL ABSTRACT



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ABSTRACT

The effect of drying (oven and freeze-drying) and the addition of maltodextrin as a carrier to Kakadu plum (*Terminalia ferdinandiana*) puree powder samples were evaluated using a combination of two dimensional (2DCOS) and near infrared (NIR) spectroscopy. Fruit powder samples were obtained from an experiment where oven and freeze-drying methods were compared together with the addition of seven levels of maltodextrin to the samples (control, 5, 7.5, 10, 15, 20 and 25% w/w). Samples were scanned using a FT-NIR instrument (Tango, Bruker, Germany) and data analysed using 2DCOS. Asynchronous and synchronous 2DCOS spectroscopy were used to analyse and interpret the effects of the method of drying and the addition of maltodextrin on the NIR spectra of the fruit samples. The utilization of 2DCOS combined with NIR spectroscopy showed how the drying method affect the NIR spectra and thus the main implications of developing an effective, quick, and easy to use protocol for determining the drying method.

1. Introduction

Kakadu plum (*Terminalia ferdinandiana* Exell, Combretaceae) is an endemic plant of Australia extremely rich in antioxidant compounds [1–5]. This fruit plum has being utilised as a traditional medicine and food by the Australian Aboriginal communities where fruits of this plant have been utilised for the cure of headaches, to

Abbreviations: KP, Kakadu plum; NIR, near infrared; 2DCOS, two-dimensional spectroscopy; PC, Principal component.

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alleviating symptoms of colds and flu and as an antiseptic [1–7]. The process of transforming a fruit puree to a dehydrated powder is made easier with the addition of a carrier [1–7]. The incorporation of such carriers may lead to the improvement in stability of natural bioactive compounds such as phenols and vitamins during further processing. The bioactive compounds are entrapped in the carrier matrix, which protects these compounds from degradation [8,9]. Carriers such as maltodextrin and inulin are used in the food industry during drying. Maltodextrin is commonly used as it is free from odour and taste and is a low-cost hydrolyzed gum, and has very good oxygen barrier properties, and has shown effectiveness in the protection of bioactive compounds such as of phenols [10,11].

Processing of the fresh or frozen whole KP fruits to intermediate value added products (puree and dehydrated powder) is a common practice by the food industry. It is well known that processing and storage of fruits and vegetables (e.g. heating) might cause losses of some bioactive compounds [12,13]. Therefore, monitoring the drying process or to develop protocols for KP fruit processing is very important for the food industry in order to maintain high quality standards. Freeze-drying, is a well-established manufacturing process and is widely used during the processing of several foods as this process gently removes water from the product [12,13]. This process stabilizes the bio compounds of the samples both chemically and physically while maintaining bioactivity. However, freeze-drying can be complex, time-consuming, and expensive, compared with oven drying [12,13].

Two-dimensional correlation spectroscopy (2DCOS) was originally developed by Noda [14–18] and involved the study of spectral changes associated with mechanical deformations (or perturbations) of the sample as a function of time [14–18]. Noda and collaborators introduced a generalised 2DCOS theory [14–18], which involved a series of spectra being recorded from one or more samples during or following perturbation. The resulting spectral data matrix enables the identification of compositional differences within and between samples where perturbations have included variables such as temperature, pressure, spatial orientation and concentration [14–18]. In this way, 2DCOS has greatly aided structural analysis, where spectral peaks were not readily distinguishable in one-dimension. Two-dimensional correlation analysis not only enhances spectral resolution, but also provides insight into variation in composition and structure [14–18]. Spreading the overlapped adjacent peaks to the second dimension results in the apparent enhancement of spectral resolution [14–18]. Based on the signs of correlation peaks, useful information, like the relative directions of band intensity changes and their sequential order along the perturbation variable can be revealed in the analysis [14–18]. In this way, 2D-COS has been found to be especially useful in identifying and classifying bands originating from different sources within the sample individually [14–18].

Vibrational spectroscopy techniques have been utilised to evaluate and monitor the effect of drying in different pharmaceutical and food matrices [19–24]. In particular, near infrared (NIR) spectroscopy has demonstrated that it can be a tool to monitor changes in the samples associated with the method of processing (oven vs freeze-drying).

The effect of drying (oven vs freeze-drying) and the addition of different levels of maltodextrin as a protective compound to the Kakadu plum puree samples were evaluated using a combination of two-dimensional (2DCOS) and near infrared (NIR) spectroscopy.

2. Materials and methods

2.1. Experimental

Commercial frozen Kakadu plum (KP) puree (ca. 15 kg) purchased from Traditional Homeland Enterprises Holding Co Pty Ltd, Morwell (Victoria, Australia) was thawed at 4 °C overnight and used for KP powder production. Maltodextrin (food grade) with DE = 17–19 from Manildra Group (Gladesville, NSW, Australia) was completely pre-dissolved in water before adding to KP puree at different levels from 5 to 25% (w/w). The blend mixtures (approx. 500 g) were homogenized for 4 min at maximum speed using a high-speed homogeniser (Ultra-Turrax® T25, IKA, Germany). The homogenous mixtures were spread onto stainless steel trays (50 × 30 cm) and subsequently subjected to either freeze drying (Lab Gear SCANVAC, QLD, Australia) at −48 °C for 7 days or conventional oven drying (Steridium, Brisbane, Australia) at 45 °C for 3 to 4 days depending on the levels of added maltodextrin. The KP puree without maltodextrin was also included as control sample. After drying, the samples were ground into a fine powder using a Laboratory blender (Waring®8010/8011, NSW, Australia). Powder samples were sieved through a 200 μm sieve to obtain uniform particle size and stored in air-tight containers at −80 °C for further analysis. All experiments were conducted in duplicate.

2.2. Water activity

The water activity was also measured in order to define the endpoint of the drying process. Approximately 2 g of the dried powder samples were placed in a standard measuring cup for measurement of water activity using a LabTouch-aw water activitymeter (Novasina AG, Labchen SZ, Switzerland) at a constant temperature of 25 ± 1 °C and average stable scanning mode. Table 1 shows the descriptive statistics for the water activity in the set of samples analysed.

2.3. Infrared spectroscopic measurements and data analysis

The FT-NIR spectra of the KP dry powder samples was collected using a Tango Bruker (Bruker Optics GmbH, Ettlingen, Germany) using a gold coated integrating sphere (diffuse reflection). Samples were placed in a cylindrical glass cuvette 10 mm diameter (Bruker Optics GmbH, Ettlingen, Germany). The reflectance spectra were recorded using OPUS software version 8.5 provided by Bruker Optics (Bruker Optics GmbH, Ettlingen, Germany) with 64 interferograms at a resolution of 4 cm^{-1} in the wavenumber range of 11,550 to 3950 cm^{-1}. Cuvettes were cleaned with 70% ethanol and dry with paper wipes between samples.

The NIR spectra was pre-processed (2nd derivative, second order polynomial, 10 smoothing points) [25] prior to 2DCOS analysis, to reduce the effect of baseline fluctuations in the raw spectra of the samples using *The Unscrambler* (version 11, CAMO, Norway). Principal component analysis was developed using The Unscrambler software, after second derivative with cross validation (full cross validation). The synchronous 2D correlation spectrum processing was subsequently performed using 2Dshige 1.3 software (Shigeaki Morita, Kwansei-Gakuin University, Japan, 2004–2005), according to the theory of generalised 2D correlation analysis developed by Noda and Ozaki [17]. In this study, synchronous and asynchronous matrices are displayed as contour maps; peaks located on the diagonal (auto peaks) show regions of the spectrum which are changing with respect to the average spectrum of the series, while peaks off the diagonal (cross peaks) show the correlation intensity (positive or negative) of different bands [17]. In this study, red and blue areas in the 2D contour maps represent positive and negative correlation intensities, respectively.

Table 1
Water activity in Kakadu plum powder samples dried using oven or freeze dry method.

	Mean	Min.	Max.	SD	CV (%)
Oven dry	0.48	0.31	0.58	0.09	18.8
Freeze dry	0.06	0.05	0.09	0.01	16.6

Min: minimum; Max: maximum; SD: standard deviation; CV: (SD/mean) × 100.

3. Results and discussion

3.1. Near infrared spectra

The NIR spectra of the KP powder samples processed either using the oven or freeze-drying methods are showed in Fig. 1. The main absorbance values were observed at 8288 cm^{-1} associated with O—H tones, around 6824 cm^{-1} (O—H stretch overtone), 5632 cm^{-1} CH_2 (cellulose), 5160 cm^{-1} O—H combination tones (water) and around 4768 cm^{-1} can be associated with both C—H and C—H_2 stretching tones, 4384 cm^{-1} and 4282 cm^{-1} might be associated with polysaccharides and cellulose [26] (see Fig. 2).

The second derivative was applied to enhance some of the features not easily observed in the raw spectra as well as to reduce the variability due to scatter. Shoulders appeared around 7544 cm^{-1} and 7480 cm^{-1} (CH_3) and around 7152 cm^{-1} and 7112 cm^{-1} associated with O—H and C—H combination tones [26]. Shifts in the spectra shoulders were observed and directly related with the drying method utilised. At 5200 cm^{-1}, two groups can be clearly observed related with the method of drying determining changes in the O—H stretch second overtone, O—H stretching and C—O stretching combinations tones, mainly associated with water [26]. Changes were also observed at 4776 cm^{-1} (O—H), 4392 cm^{-1} (CH_2) and 4288 cm^{-1} related to O—H bending and C—O stretching combinations, C—H stretching and CH_2 deformation combination tones [26]. These bands can be associated with the typical composition of food matrices containing different levels of sugars and polysaccharides (e.g. cellulose, starch). In addition, samples with high levels of maltodextrin added to the matrix showed slight differences in bands at the wavelength ranging between 5001 and 5251 cm^{-1} and 5251 cm^{-1} to 6101 cm^{-1} associated with O—H and C—H matching the NIR spectra of pure maltodextrin [26].

Three isosbestic points were observed between the KP samples dried using the two methods. These points were located at 8680 cm^{-1}, 6472 cm^{-1} and 6170 cm^{-1} [26]. However, it has been also observed a small shift in the isosbestic point due to the addition of maltodextrin to the KP powder samples. This spectral shift can be explained by the fact that maltodextrin can create a film entrapping the molecules in the matrix and thereby acting as a protective agent against oxidation of some of the main compounds in the puree samples. This is having a direct effect on the absorption of compounds such as sugars, phenolic (e.g. aromatic ring) and polysaccharides that will also contribute to the observed differences in the absorbance values.

Water activity was also measured in all the powder samples analysed. It is well known that residual moisture content contributes to the quality attributes of a sample. This residual moisture content should be as low as possible to minimize any chemical or physical degradation of the sample. It has been observed (see Table 1) that freeze dried samples had similar and consistent moisture content and water activity compared with the oven dried samples. This trend can be observed in the wavelength around 6990 cm^{-1}. This indicated that the freeze-drying method yielded the samples with very similar levels of residual moisture.

3.2. Principal component analysis

Principal component (PC) analysis was utilised to interpret the effects of the drying method and the addition of maltodextrin to the NIR spectra of the KP powder samples analysed. Fig. 3 shows the PC scores plot for the NIR spectra of the KP powder samples using the two drying methods (effect of drying). For this purpose, the controls and high level of maltodextrin added to the samples in each of the drying method were also compared (direction of the arrows). The direction of the arrows highlighted the effect of the addition of maltodextrin to the puree samples. The first two principal components (PC1 85% and PC2 14%) explained 99% of the variability in the NIR data set. The scores plot showed a clear trend in the set of samples related with the effect of drying and the addition of maltodextrin to the KP plum powder samples analysed using NIR spectroscopy. The loadings plot (data not presented) indicated that the wavelengths derived from the first PC were closely related with the method of drying while the second PC explained the changes associated with the addition of mal-

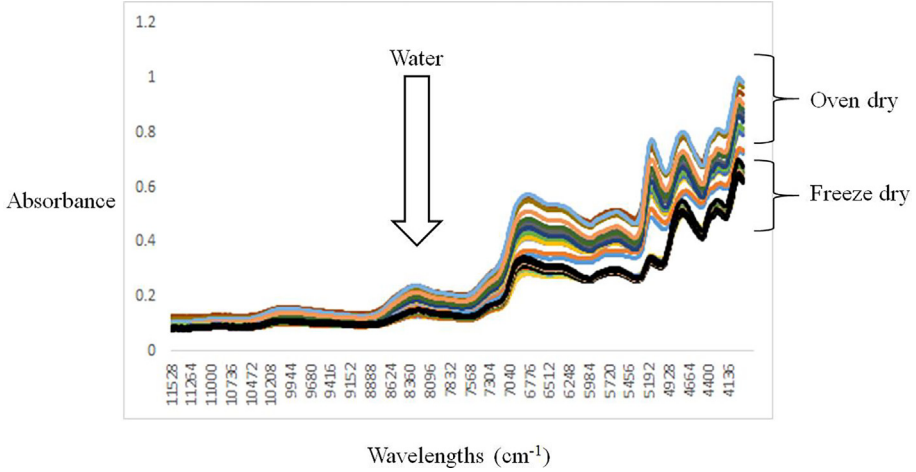

Fig. 1. Near infrared spectra of Kakadu plum powder samples dried using freeze drying or oven drying methods.

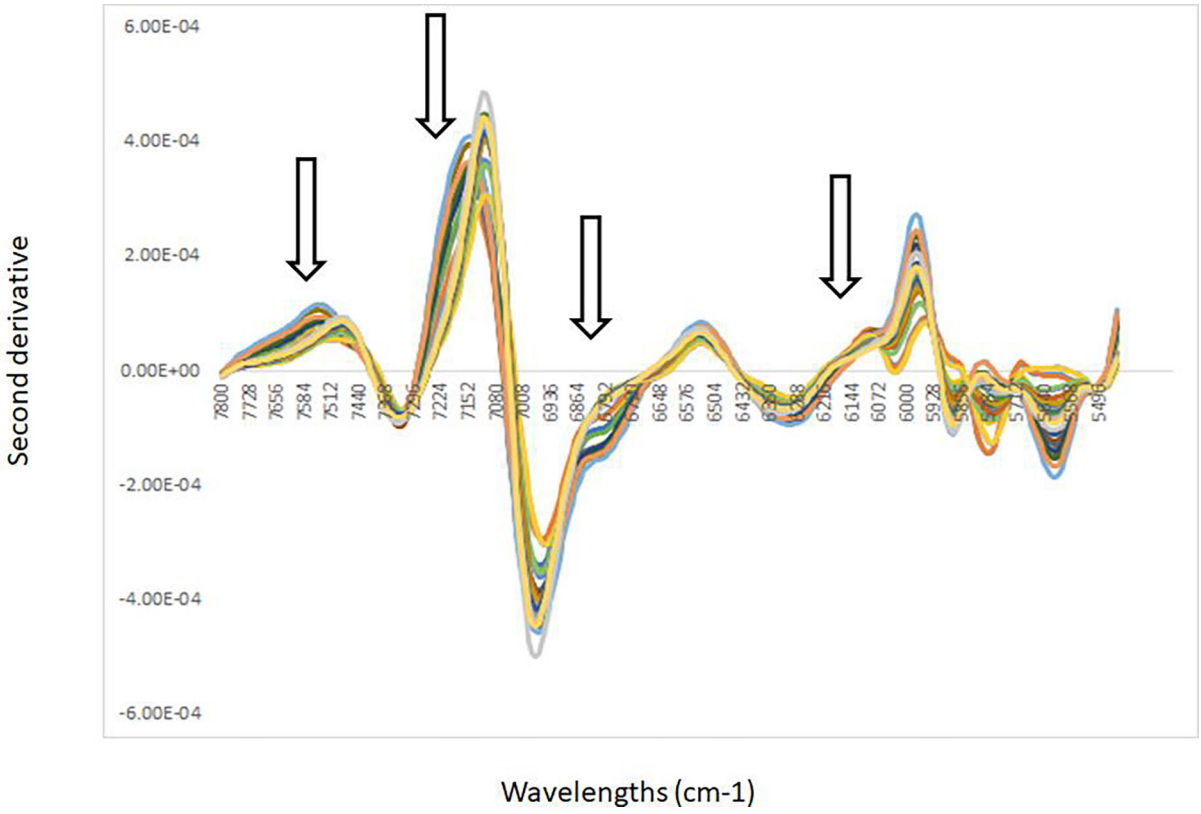

Fig. 2. Second derivative of the near infrared spectra of Kakadu plum powder samples dried using freeze drying or oven drying method. Arrows indicated the position of the shifts in the spectra due to processing.

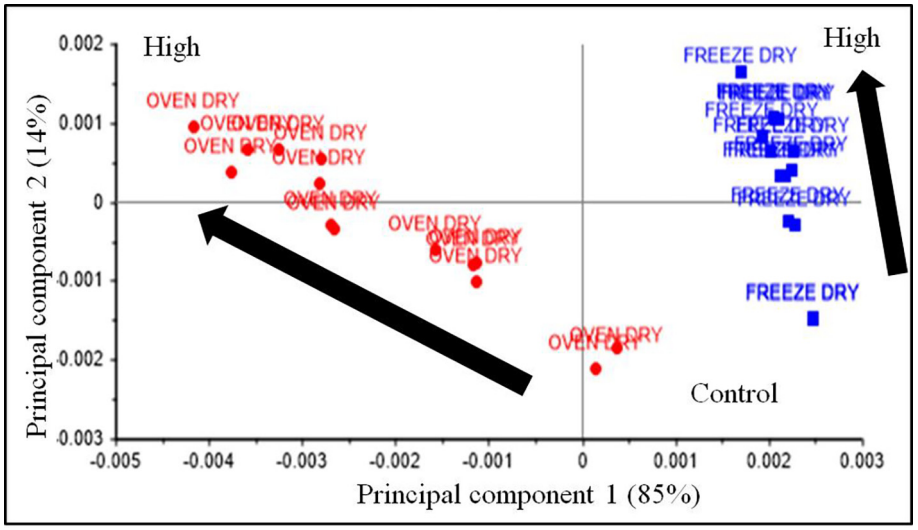

Fig. 3. Principal component score plot of Kakadu plum powder samples analysed using near infrared spectroscopy. The direction of the arrows indicated the increase level of maltodextrin.

todextrin to the KP powder samples. Shifts were also observed in the loadings around 7200 cm^{-1}, 5880 cm^{-1} and in the region between 4900 and 4200 cm^{-1} corresponding with the band assignations described above [26].

3.3. Two dimensional spectroscopy

Compared with the mathematical enhancement of the spectral resolution using derivatives where 2DCOS can enhance the spectral resolution associated either with physical or chemical perturbations [14–18]. For example, using temperature as the perturbation (drying method), underlying peaks in an envelope band may be resolved when different components show different responses to the drying method [14–18]. The results of this research are based on the thermal perturbations derived from the use of two different drying methods and the addition of maltodextrin to the samples to generate 2DCOS spectra of the KP fruit powder samples for the further evaluation of the treatments.

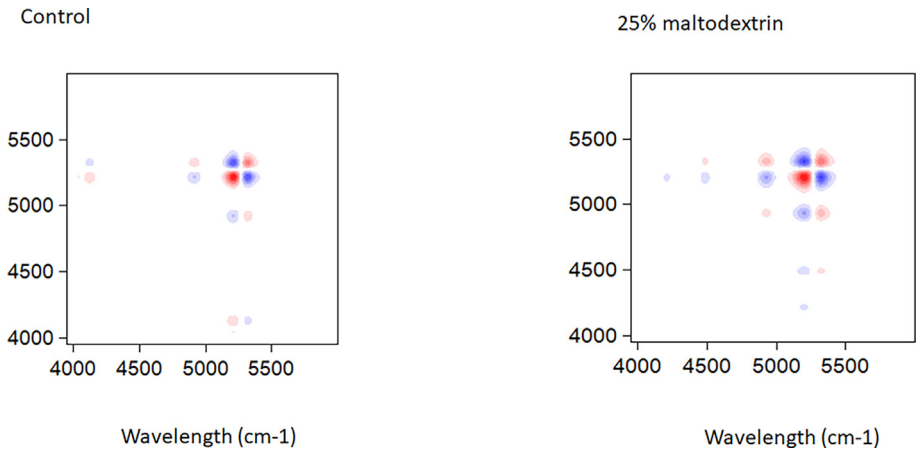

Fig. 4. Synchronous 2DCOS of the KP control samples and with the addition of 25% malrodextrin dried by oven and freeze-drying analysed suing near infrared spectroscopy.

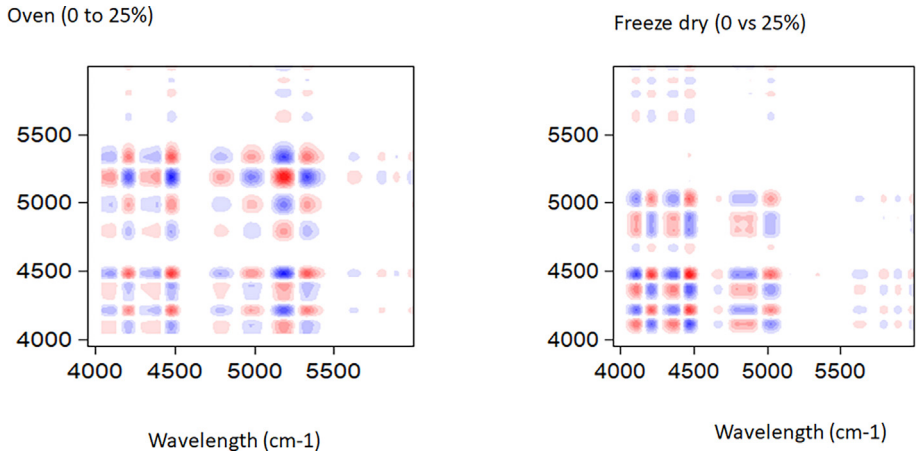

Fig. 5. Synchronous 2DCOS of oven and freeze dry samples analysed suing near infrared spectroscopy.

The synchronous 2D correlation NIR spectrum shows the overall similarity or simultaneous changes of two independent dynamic spectra over the interval between the two drying methods (oven and freeze-drying) using the control samples. Figs. 4 and 5 show the 2DCOS NIR synchronous correlation spectra of the KP powder samples processed using oven and freeze dry methods. In particular, Fig. 4 shows the synchronous of the KP control samples and the samples with the high levels of maltodextrin added to the matrix (25%) comparing to the drying method (oven vs freeze-drying). It was observed that absorption bands at 5320 cm^{-1} have a relatively intense positive correlation with the 5240 cm^{-1} and assigned to the O—H (water) and C═O and C—H combination vibrations [26] while negative correlations were observed between the bands 5320 and 4128 cm^{-1} (aromatic groups) [26]. The observed negative correlation can be associated with the effect of drying to the fruit puree samples. It has been also observed that the synchronous NIR spectra contain additional positive and negative cross-peaks. This positive cross-peak in the synchronous correlation spectrum shows that the bands creating the cross-peak co-varies (originated from the same molecular vibrations), whereas a negative cross-peak associated with those bands sharing the cross-peak are inversely related and should have different origins. Some degree of correlation with specific NIR wavelengths in the spectra associated can be observed between water and sugars (5320 and 4130 cm^{-1}) [26].

Fig. 5 shows the 2DCOS synchronous of the KP powder samples having the increase addition of maltodextrin (control to 25% w/w) in each of the drying methods (oven or freeze dry). Different to the data presented in Fig. 4 that the drying method was analysed, the data in Fig. 5 shows the effect of the addition of different levels of maltodextrin into the KP puree samples. It was observed that absorption bands at 5032 cm^{-1} (N—H amide groups), 4440 cm^{-1} (C—H), 4370 cm^{-1} (C═O and aromatic groups), 4200 cm^{-1} (C—H stretching and combinations) and 4100 cm^{-1} (aromatic groups) have relatively intense positive correlation with the 5240 cm^{-1} and this is assigned to the O—H (water) and C═O and C—H combination vibrations [26] while negative correlations were observed between the band 5320 cm^{-1} (C—H) and 4128 cm^{-1} (aromatic groups). The observed negative correlations can be associated with the effect of maltodextrin to the fruit powder samples.

Likewise, to the data presented in Fig. 4, the synchronous NIR spectra contain extra positive and negative cross-peaks. In this case, cross-peaks associated with water and polysaccharides are a strong correlation with other wavelengths in the NIR region. Moreover, a negative cross peak was observed in the NIR region between wavelengths associated with water (O—H) and other compounds such as polysaccharides (e.g. cellulose, starch) [26]. The existence of a negative cross peak between the water bands those in the NIR region associated with C-H groups might suggest that these bands can be related with the presence of changes and interaction in C—H and O—H bonding as consequence of the method of drying and addition of maltodextrin. In this study, the use of 2DCOS spectroscopy allowed to monitor changes in the KP

powder samples associated with the drying method as well as the interactions with the addition of maltodextrin as a protective agent.

4. Conclusion

The combination of 2DCOS with NIR spectroscopy illustrated how the drying method applied to process the samples affect the NIR spectra of KP samples and thus the main implications of developing protocols for efficiently processing samples by the industry. This study has demonstrated that freeze dried samples had similar and consistent moisture content compared with the oven dried samples (absorbance around 6990 cm^{-1}). These findings indicated that the freeze dry method yielded samples with very similar levels of residual moisture. Overall, this study is providing with a low-cost and rapid methodology to assess the drying process of KP samples. This method can be easily implemented by the industry.

CRediT authorship contribution statement

Daniel Cozzolino: Conceptualization, Formal analysis, Validation, Writing - review & editing. **Anh Dao T. Phan:** Conceptualization, Formal analysis, Resources. **Michael Netzel:** Writing - review & editing. **Heather Smyth:** Writing - review & editing. **Yasmina Sultanbawa:** . **:** Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The Use of a Micro Near Infrared Portable Instrument to Predict Bioactive Compounds in a Wild Harvested Fruit—Kakadu Plum (*Terminalia ferdinandiana*)

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Abstract: Kakadu plum (KP; *Terminalia ferdinandiana* Exell, Combretaceae) is an emergent indigenous fruit originating from Northern Australia, with valuable health and nutritional characteristics and properties (e.g., high levels of vitamin C and ellagic acid). In recent years, the utilization of handheld NIR instruments has allowed for the in situ quantification of a wide range of bioactive compounds in fruit and vegetables. The objective of this study was to evaluate the ability of a handheld NIR spectrophotometer to measure vitamin C and ellagic acid in wild harvested KP fruit samples. Whole and pureed fruit samples were collected from two locations in the Kimberley region (Western Australia, Australia) and were analysed using both reference and NIR methods. The standard error in cross validation (SECV) and the residual predictive deviation (RPD) values were 1.81% dry matter (DM) with an RPD of 2.1, and 3.8 mg g^{-1} DM with an RPD of 1.9 for the prediction of vitamin C and ellagic acid, respectively, in whole KP fruit. The SECV and RPD values were 1.73% DM with an RPD of 2.2, and 5.6 mg g^{-1} DM with an RPD of 1.3 for the prediction of vitamin C and ellagic acid, respectively, in powdered KP samples. The results of this study demonstrated the ability of a handheld NIR instrument to predict vitamin C and ellagic acid in whole and pureed KP fruit samples. Although the RPD values obtained were not considered adequate to quantify these bioactive compounds (e.g., analytical quantification), this technique can be used as a rapid tool to screen vitamin C in KP fruit samples for high and low quality vitamin C.

Keywords: near infrared; vitamin C; ellagic acid; wild harvest; Kakadu plum

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1. Introduction

Kakadu plum (KP; *Terminalia ferdinandiana* Exell, Combretaceae) is an emerging indigenous fruit originating from Northern Australia, with valuable health and nutritional characteristics and properties such as high levels of vitamin C, ellagic acid, and other polyphenolic compounds [1–6]. Kakadu plum is the most common name for this fruit, and it is found from the Kimberley (Western Australia) to Darwin (Northern Territory) and Queensland regions [1–6]. Commercial harvesting of KP fruit started in the late 1990s. While the vast majority of production is from wild harvested fruit [1–6], some commercial orchards can be found in Australia. Like many wild-harvested native foods, weather conditions, including drought, bushfires, and cyclones, might have an impact on the volume of fruit available, so production is highly variable from year to year [1–6]. The main harvest time is January, although some trees have multiple flowerings and can produce fruit up until July, depending on the region. The production of this fruit is estimated to average

15–17 tonnes per annum [1–6]. Although the KP is commercialised as whole fruit, it can be processed as a pureed or dehydrated powder (e.g., freeze dried) [7]. The dehydrated powder is used as a functional food ingredient in order to add value to a wide range of different food products (e.g., yogurts and ice creams), a common practice in the food industry [1–7]. It is well recognised that the health benefits of native plants are attributed to the content of antioxidant compounds such as natural ascorbic acid (vitamin C) and polyphenols, including gallic and ellagic acids [4,5]. These antioxidants have become very important in human health and nutrition, motivating the rapidly expanding search for plant sources containing these compounds in the wild (e.g., native plants). Kakadu plant materials (e.g., fruit and leaves) have high quantities of ellagic acid, together with the bioactive forms of vitamin C (ascorbic acid), making this plant very attractive as a source of natural antioxidants [4–6].

In recent years, applications based on the use of vibrational spectroscopy (near infrared, mid infrared, and Raman) have been utilised to quantify and monitor the composition and nutritional value in a wide range of plant and fruit materials [8–13]. In particular, the use of near infrared (NIR) spectroscopy has demonstrated that it can be a versatile tool to analyse different types of samples and conditions [8–13]. These recent developments in portable and handheld instrumentation have opened a new window for utilising these types of instruments to analyse and monitor the composition of fruit and vegetables [8–14]. In this context, the utilization of handheld instrumentation is allowing the quantification of antioxidants and bioactive compounds in native or wild harvest fruit samples like KP fruit.

Therefore, the objective of this study was to evaluate the ability of a handheld NIR instrument combined with chemometrics to measure vitamin C and ellagic acid concentrations in KP fruit samples.

2. Materials and Methods

2.1. Samples

Kakadu plum fruit samples were wild harvested in January 2020 from two different locations in the Kimberley region (Western Australia, Australia). Ten KP trees from each site were randomly selected for harvesting (approximately 50–100 fruit per tree). The samples were stored and transported to the laboratory under refrigerated conditions and then immediately stored at −80 °C for further analysis. The frozen fruit samples were thawed at room temperature (20 °C) before the NIR and reference analyses. After NIR scanning, the fruit samples were blended into a puree using a mortar and pestle. Consequently, the obtained pureed samples were analysed as a puree using the same NIR spectrophotometer as described in the section below (infrared spectroscopic measurements). Following the NIR analysis, the pureed samples were lyophilized (Lindner and May Ltd., Windsor, QLD, Australia) and finely ground using a Retsch MM301 cryomill (Retsch GmbH, Haan, Germany) in order to provide a uniform powder for the determination of vitamin C and ellagic acid. After all of the fruit samples underwent NIR scanning, representative samples were selected using principal component analysis in order to be utilised for further reference analysis and calibration development.

2.2. Infrared Spectroscopic Measurements

The NIR spectra of either whole (n = 60) or pureed (n = 60) KP fruit samples were collected using a portable NIR spectrophotometer (Micro-NIR 1700, Viavi, Milpitas, CA, USA) operating in a 950–1600 nm wavelength range, with a spectral resolution of 10 nm with no moving parts (Viavi Solutions, 2015, Milipitas, CA, USA). The NIR instrument was connected through a USB interface to a notebook computer running proprietary software (MicroNIR Prov 3.1, Viavi, Milpitas, CA, USA) for the acquisition of the diffuse reflectance spectra of the samples (Viavi Solutions, 2015, Milipitas, CA, USA). The controlling parameters for the spectral data acquisition were set at 50 min integration time and with an average of 50 scans (MicroNIR Prov 3.1, Viavi, Milpitas, CA, USA). The reference spectra for the

absorbance/reflectance calculations were collected using Spectralon® after the consecutive scanning of 10 samples.

2.3. Determination of Ellagic Acid

The extraction and analysis of ellagic acid (EA) were conducted according to the method previously reported by Williams and collaborators, with some modifications [5,6]. Briefly, 100 mg of powdered samples were extracted with 80% methanol containing 0.01N HCl using a vortex, followed by sonication for 10 min. The free EA released in the supernatant (referred to as extract A) was collected after being centrifuged (3220× *g*, 5 min at 20 °C; Eppendorf Centrifuge 5810 R, Hamburg Germany), whereas the residues were extensively extracted with absolute methanol in order to completely release the remaining free EA (extract B).

In order to measure the EA existing under bound form (e.g., ellagitannins), hydrolysis was conducted following the method reported by Williams and collaborators [5,6]. The obtained extract A was added into a 5 mL Reacti-Therm vial (Fisher Scientific, Bellefonte, PA, USA) and subjected to overnight hydrolysis at 90 °C using 2N HCl. The EA released after hydrolysis was dissolved in methanol (referred to as extract C) before the UPLC-PDA analysis.

EA in three different extracts was analysed using a Waters AcquityTM UPLC-PDA System (Waters, Milford, MA, USA). The compound was separated on a Waters BEH Shield RP C18 column (100 × 2.1 mm i.d; 1.7 µm) maintained at 35 °C. The mobile phases included 0.1% formic acid (FA) in Milli-Q water (A) and 0.1% FA in methanol (B). The flow rate was 0.3 mL/min, with the following gradient elution for B: 35% B isocratic conditions for 5 min, 50% B for 10 min, and 100% B for 15 min. The contents of free EA (extracts A and B) and total free and bound EA (extracts B and C) were quantified using an external calibration curve of ellagic acid acquired at 254 nm [5,6].

2.4. Determination of Vitamin C

The extraction and analysis of vitamin C in the powder samples were conducted following a method previously described elsewhere [15]. Briefly, 100 mg of powdered KP samples were extracted with 3% meta-phosphoric acid containing 8% acetic acid and 1 mL ethylenediaminetetraacetic acid (EDTA). The reduction of dehydroascorbic acid (DHAA), which was also present in the extracts/samples, to ascorbic acid (L-AA) was performed [15,16]. The total vitamin C (L-AA + DHAA) was determined using a Waters UPLC-PDA system and a Waters HSS-T3 column (150 × 2.1 mm i.d; 1.8 µm; 25 °C), with water with 0.1% formic acid as the mobile phase (0.3 mL/min) under isocratic elution. Vitamin C was quantified using an external calibration curve of ascorbic acid acquired at 245 nm [15].

2.5. Data Analysis

The NIR spectra were pre-processed (second derivative, second order polynomial, 21 smoothing points) using The Unscrambler software (version 11, CAMO, Oslo, Norway) [17]. A principal component analysis was conducted using The Unscrambler software, after a second derivative with cross validation (full cross validation) [18]. Partial least squares regression (PLS) was used to relate the NIR spectra with the content of vitamin C and ellagic acid in the KP fruit samples analysed. To evaluate the performance of the PLS models, validations were performed on two different datasets. For the purpose of this study, the original dataset was split into two subsets of 70% (e.g., calibration) and 30% (e.g., validation), using the Kennard-Stone algorithm [19]. Thus, 40 uniformly distributed samples were selected and used in the calibration, while 20 samples were used for validation. By performing data partitioning, knowledge of the training dataset did not affect the test dataset, and the predictive power of the created model subsequently increased. Leave-one-out cross-validation was applied on the calibration set for internal validation, and the test set was used to externally validate the generated models. The coefficient of determination

(R^2), the standard error in cross validation (SECV), and the residual predictive deviation (RPD) were used to evaluate the calibration models developed [18,20–22].

3. Results and Discussion

Table 1 reports the descriptive statistics (e.g., average, standard deviation, range, and coefficient of variation) for the measurement of the dry matter, vitamin C, and ellagic acid content in the KP fruit samples used to develop the NIR calibrations. Table 2 shows the cross validation and validation statistics for the prediction of vitamin C and ellagic acid in the set of whole and pureed KP fruit samples analysed. As stated above, the SECV and the RPD (SD/SECV) were used to evaluate the ability of the PLS models developed to predict these parameters [18,20]. SECV is a quantitative measure of how precise the samples are predicted during validation where the bias is a systematic deviation of the predicted values from the true value due to a particular measurement method [18,20]. The SECV and RPD values were 1.81% dry matter (DM) with an RPD of 2.1, and 3.8 mg g^{-1} DM with an RPD of 1.9 for the prediction of vitamin C and ellagic acid, respectively, in the set of whole KP fruit samples. Using the set of pureed KP samples, the SECV and RPD values were 1.73% DM with an RPD of 2.2, and 5.6 mg g^{-1} DM with an RPD of 1.3 for the prediction of vitamin C and ellagic acid, respectively. According to other authors, an RPD value between 2 and 2.5 might indicate that rough quantitative predictions could be possible, while a value between 2.5 and 3 or above might be associated with good and excellent prediction accuracy [18,20–23]. The RPD values in this study were between 1.3 to 2.2 for the prediction of vitamin C and ellagic acid. Similar SECV values were reported by other authors using mid infrared spectroscopy to predict ellagic acid in coastal oak samples [24].

Table 1. Descriptive statistics for the measurement of vitamin C and ellagic acid in Kakadu plum fruit samples analysed using NIR spectroscopy.

	% DM	VIT C (% DM)	EA (mg g^{-1} DM)
Average	16.4	12.5	20.64
SD	1.2	3.81	7.7
Minimum	14.2	7.8	7.6
Maximum	18.7	19.3	31.5
CV (%)	7.3	30.4	37.4

CV—coefficient of variation (CV = SD/mean); DM—dry matter; EA—total ellagic acid; SD—standard deviation; VIT C—vitamin C.

Table 2. Cross validation and validation statistics for the prediction of ellagic acid and vitamin C in whole and pureed Kakadu plum sample analyses using near infrared reflectance spectroscopy.

		R^2_{CV}	SECV	Slope	Bias	LV	RPD_{CV}	r	SEP
Whole	VIT C (% DM)	0.55	1.81	0.53	0.029	8	2.1	0.85	2.0
	EA (mg g^{-1} DM)	0.57	3.8	0.61	−0.007	11	1.96	0.55	7.5
Puree	VIT C (% DM)	0.86	1.73	0.87	0.10	8	2.2	0.89	1.9
	EA (mg g^{-1} DM)	0.48	5.6	0.57	0.002	11	1.3	0.56	6.2

CV—cross validation; DM—dry matter; LV—number of optimal latent variables used to develop the models; VIT C—vitamin C; EA—total ellagic acid; R^2CV—coefficient of determination in cross validation; r—correlation coefficient in prediction; RPD—SD/SECV; SECV—standard error for cross validation; SEP—standard error of prediction.

R^2 indicates the percentage of variance present in the true component values, which will be reproduced in the prediction (18, 20–23). Depending on the R^2 values obtained during the calibration process, the NIR models can be classified as follows: possessing a low correlation ($0.26 < R^2 < 0.49$), models that can be used to discriminate between a low and high composition of samples ($0.50 < R^2 < 0.64$), models that can be used for a rough prediction of the composition ($0.65 < R^2 < 0.81$), possessing a good correlation ($0.82 < R^2$

< 0.90), and having excellent precision (R^2 > 0.90) [18,20–23]. The PLS calibration models developed using the pureed KP samples explained between 48% and 86% of the variation related to vitamin C and ellagic acid, while 55% to 57% of the variation was explained in the calibration models using the whole KP fruit samples. The observed differences in the PLS models were associated with sample presentation (whole vs. pureed fruit).

It has been reported that the NIR spectra are comprised of wide bands originating from overlapping absorptions corresponding to overtones and combinations of vibrational modes involving C-H, O-H, and N-H chemical bonds [8,25,26]. Although the water absorption bands related to the O-H bonds are predominant in the NIR spectra of fruit such as KP fruit, other molecules can be measured [8,25,26]. Carbohydrates, organic acids, proteins, and other minor compounds can exhibit wide absorption bands as a result of complex hydrogen bonding interactions with different molecules in the NIR wavelength range [8,24,25]. Therefore, the interpretation of the NIR spectra is not as straight forward as the interpretation of the MIR region [8,25,26].

In order to understand the basis of the NIR calibrations developed, the PLS loadings were analysed and interpreted for each of the sample presentations used to develop the calibrations for vitamin C and ellagic acid (e.g., whole or pureed fruit; Figures 1 and 2). The relationships between the wavelength and PLS latent variables/loadings imply that these wavelengths contribute to explaining the developed models [18,20–22]. Therefore, the value and direction (e.g., positive and negative) of the PLS loading indicated the contributions of individual wavelengths to the model [18,20–22]. It has been reported that when PLS models are developed for the same parameters, using different pre-processing or sampling presentation modes for the same sample, they can utilise different wavelengths or loadings. In this study, sample presentations (whole vs. pureed) were shown to have an effect by explaining the observed differences in the PLS calibrations and loadings. The loadings used by the PLS calibrations for the measurement of vitamin C and ellagic acid in the KP puree samples are shown in Figure 1. The loadings for vitamin C were observed at wavelengths of around 1137 nm (C-H combination, aromatic groups), 1217 nm (C-H_2), 1299 nm (first overtone of C-H combination), 1465 nm (N-H associated with secondary amines), and 1558 nm (O-H), whereas for ellagic acid, the most important wavelengths were observed at 1174 nm (C-H), 1310 nm (first overtone of C-H combination), 1410 nm (O-H bonds), and 1510 nm (N-H amide) [8,25–33]. The PLS loadings observed for the calibrations developed using the whole KP fruit samples are shown in Figure 2. The main loadings were observed at 1093 nm (C-H, aromatic groups), 1347 nm (C-H), 1465 nm (N-H), and 1570 nm (N-H) for vitamin C, while for ellagic acid, four wavelengths were observed to influence the models, at 1155 nm (C-H), 1242 nm (C-H), 1440 nm, and 1508 nm (C-H and N-H) [8,25–33]. It has been observed that the calibration models for the same parameters used similar wavelengths, and these might indicate that the sample presentation (whole vs. pureed fruit samples) might not have a greater effect on the information collected by the NIR instrument for the prediction of the bioactive compounds in the set of KP fruit samples analysed.

Figure 3 shows the scatter plot for the validation of the measurement of vitamin C and ellagic acid in the pureed samples. The influence of the region or origin of the samples was observed upon cross-validation models developed for vitamin C (bimodal distribution as a result of region). However, this trend was not observed for the prediction of ellagic acid in the KP fruit samples analysed. In addition, one and three outlier samples were observed in the prediction of vitamin C and ellagic acid, respectively. A detailed analysis of these outlier samples indicated that they corresponded to spectral outliers. These results are in agreement with those reported by other authors, who indicated that region might have an effect on the concentration of some of these bioactive compounds [1–3,7]. It is well known that vitamin C is an important parameter because of its important health and antioxidant properties, which have received a great deal of attention, thus necessitating the development of rapid analytical methods [26]. However, some authors have reported unsatisfactory results using short wavelengths in the NIR region or when the samples

contain low concentrations of vitamin C (less than 10 g L^{-1}) [22,28–33]. Another reported issue might be related to the effect of moisture and its interference when determining the presence of compounds with low concentrations. Recently, Oliveira-Folador and collaborators [33] suggested that the high water content of the pulp of fruit (approximately 84%) contributes to the inherent complexity of NIR spectra. This might also be explained by the fact that the NIR spectral range used is highly sensitive to elements that modify light diffusion, such as physical structure and the presence and content of water in the sample [20,21]. The physical structure of the fruit has been reported to have a large effect on the acquisition of spectra, and this is strongly influenced by the light scattering phenomena, as reported by other authors in different types of fruit and vegetables [22,28–32]. Figure 4 shows the principal component score plot of the KP samples scanned as whole and pureed fruit. Two groups were observed related to the sample presentation used. Whole samples tended to scatter along principal component one (50% of the variation), while most of the pureed samples were clustered together. It is also important to highlight that the NIR spectrum of fresh materials is essentially composed of a large set of overtones and combination bands. This combination, together with the complex chemical composition of a typical fruit or vegetable, makes the near infrared spectrum highly complex [8,25]. Regardless of these issues, the NIR region used in this study showed a high applicability for the rapid screening of samples for high, medium, and low vitamin C and ellagic acid.

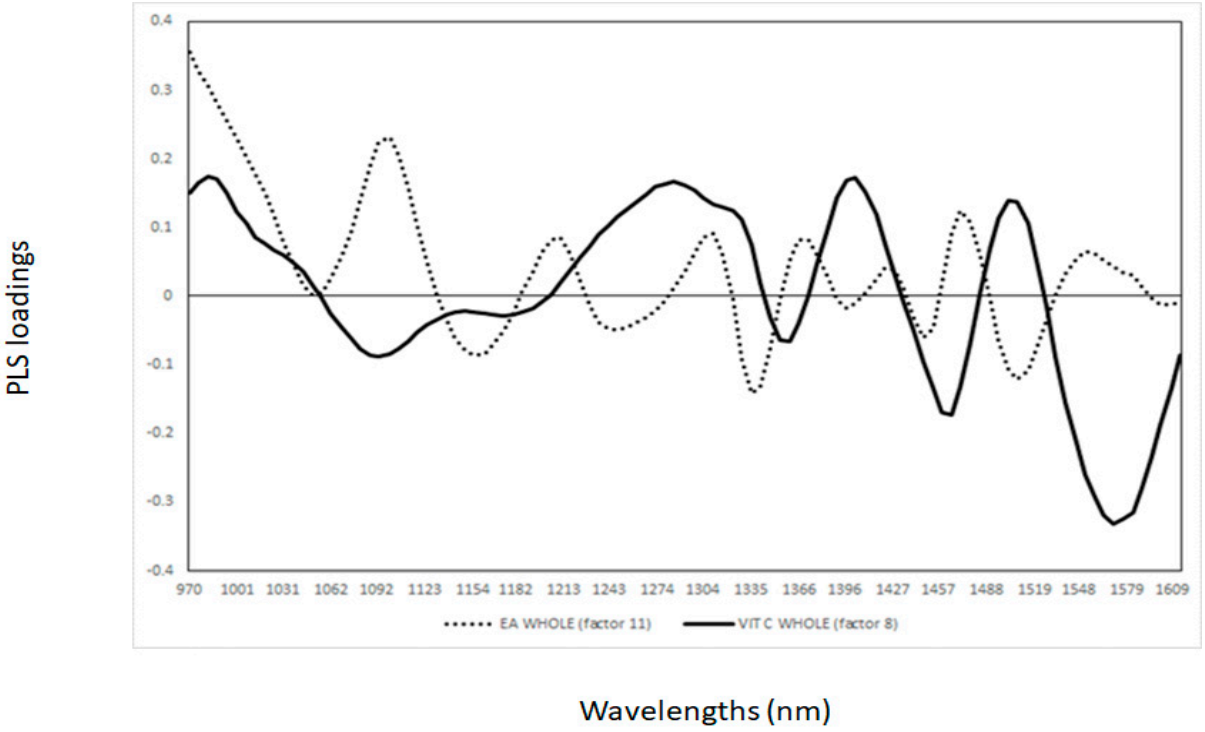

Figure 1. Partial least square loadings for the measurement of vitamin C and ellagic acid in whole Kakadu plum fruit samples.

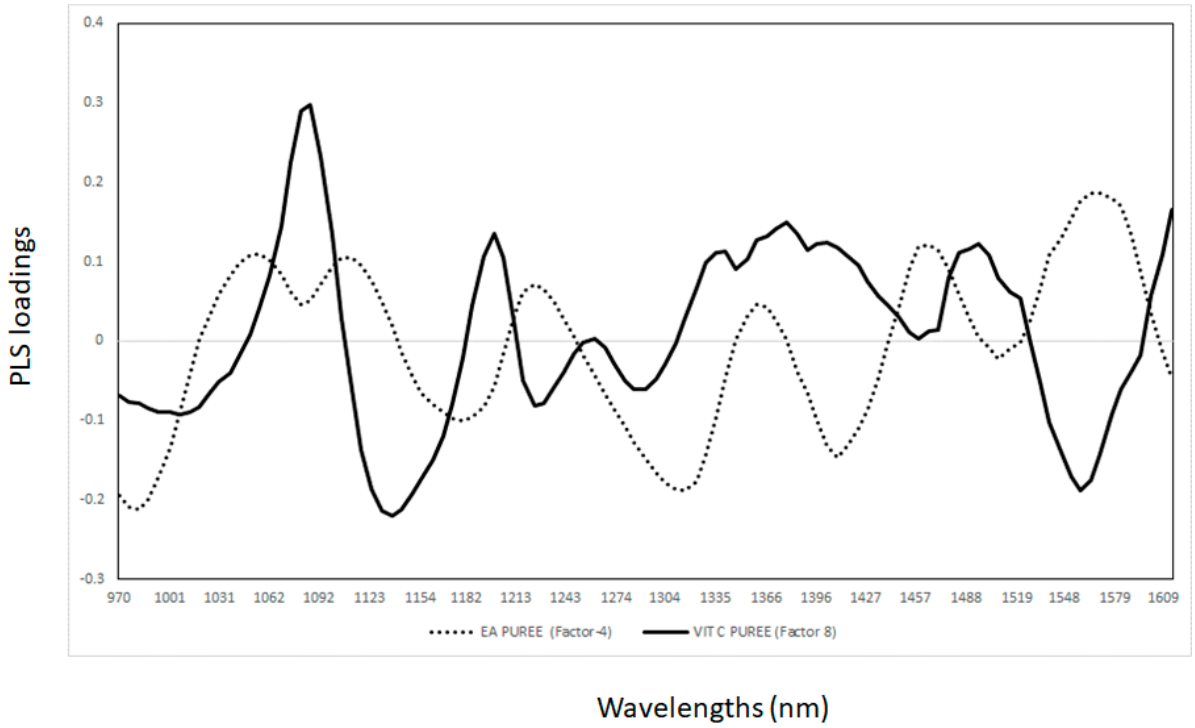

Figure 2. Partial least square loadings for the measurement of vitamin C and ellagic acid in pureed Kakadu plum fruit samples.

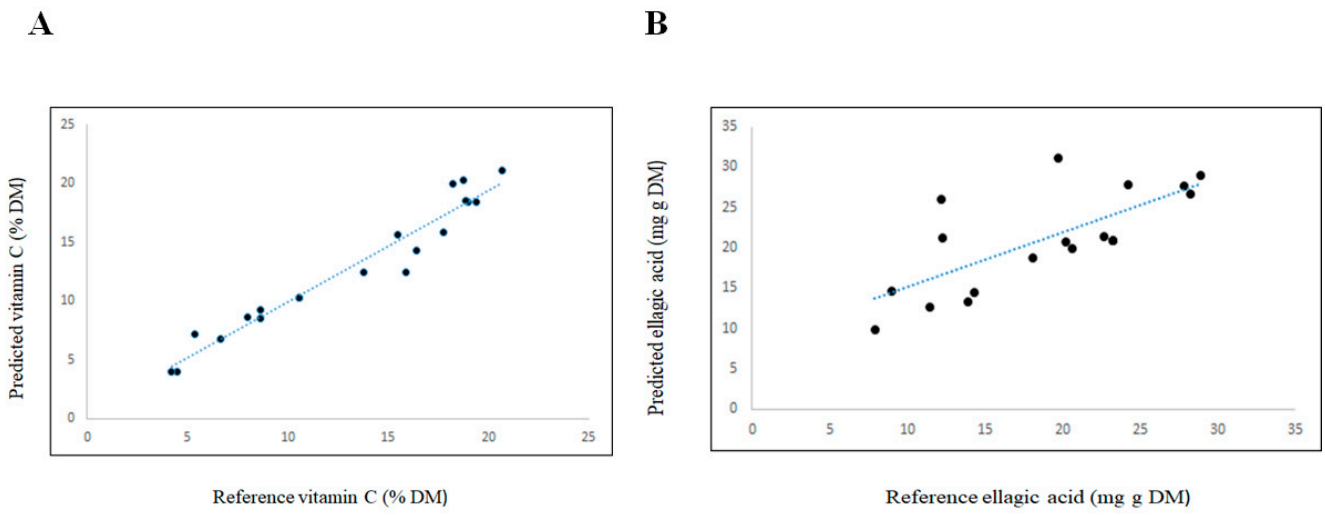

Figure 3. Scatter plot for the validation (n = 20) of the measurement of vitamin C (Panel (**A**)) and ellagic acid (Panel (**B**)) in the pureed Kakadu plum samples.

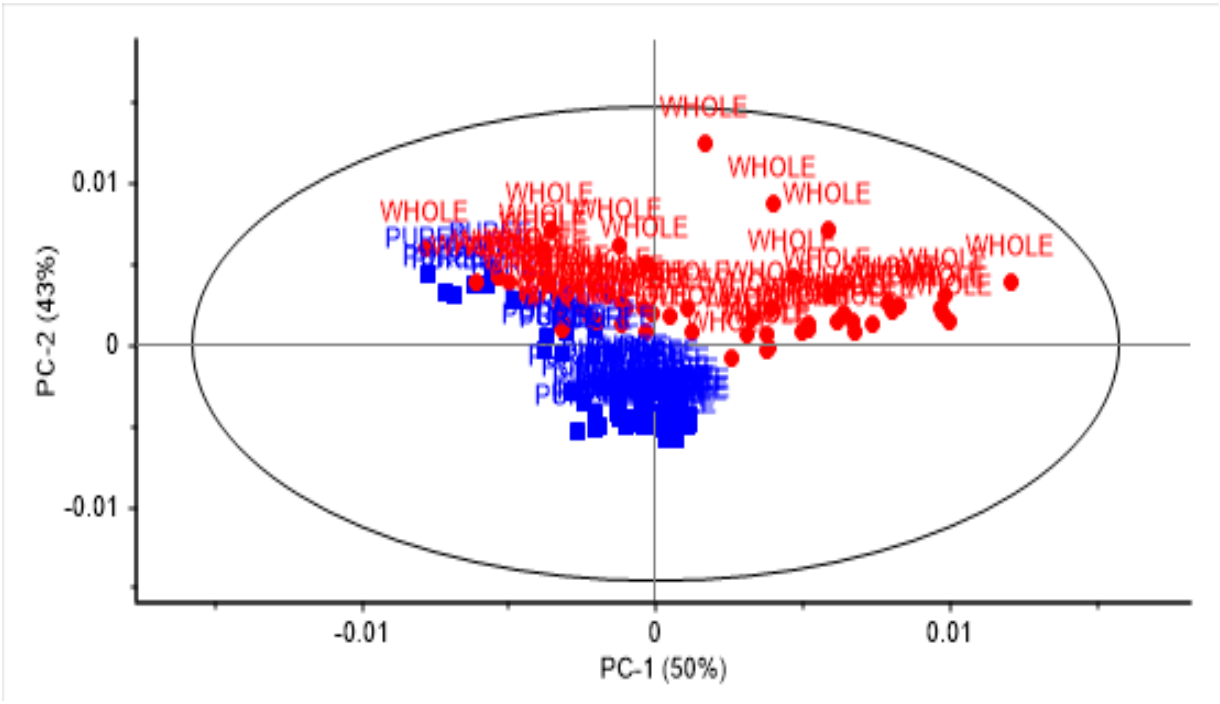

Figure 4. Principal component score plot of Kakadu plum samples analysed as pureed or as whole fruit using near infrared spectroscopy.

4. Conclusions

The results of this study showed the ability of a handheld NIR instrument to predict vitamin C and ellagic acid in both whole and pureed KP fruit samples. Although the RPD values obtained are not considered adequate to quantify these bioactive compounds, they can be used to quickly screen the fruit for high- and low-quality vitamin C. The handheld instrument used in this study can be an alternative for rapid and throughput screening of raw materials in remote areas, where it might not be appropriate to use other types of instruments to assess fruit quality (e.g., bioactive compounds). However, further studies are needed to optimize the prediction models for these bioactive compounds and to evaluate the effect of region/origin and harvest (years) in order to make the models more robust for routine applications.

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Can Infrared Spectroscopy Detect Adulteration of Kakadu Plum (*Terminalia ferdinandiana*) Dry Powder with Synthetic Ascorbic Acid?

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Abstract

Kakadu plum (*Terminalia ferdinandiana*) fruit is characterised by its high levels of natural ascorbic acid compared with other domesticated plants in the world (e.g. more than 75 times that of oranges). The concentration of ascorbic acid is one of the main factors that define the price of this fruit (as fresh or dry powder) in the market. As many other specialty foods and commodities, this food is not exempt against adulteration. Adulteration can be simply performed by the addition of water (dilution), low-quality fruit and synthetic ascorbic acid and other chemical products. The ability of attenuated total reflectance mid infrared spectroscopy (ATR-MIR) was evaluated as a tool to detect the level of adulteration of Kakadu plum powder with a synthetic source of ascorbic acid. The coefficient of determination (R^2) and the standard error of cross validation (SECV) obtained for the prediction of level and source of adulteration were 0.85 (3.2%), and 0.83 (0.30%) respectively. This study demonstrated that the integration of ATR-MIR spectroscopy with chemometric analysis could be a valuable method to identify the adulteration of Kakadu plum powder with synthetic sources of ascorbic acid.

Keywords Ascorbic acid · Infrared · Machine learning · Adulteration

Introduction

Kakadu plum (*Terminalia ferdinandiana*) (KP) is found naturally in open woodlands across the Northern-Western territories of Australia, in particular in the Kimberley region of Western Australia and the Northern Territory (Graham and Hart 1997; Hegarty et al. 2001; Netzel et al. 2007; Konczak et al. 2009; Gorman et al. 2020). Commercial harvest of KP fruit commenced in the late 1990s and requires government permits, where the vast majority of the production is sourced from wild harvest fruit (Graham and Hart 1997; Hegarty et al. 2001; Netzel et al. 2007; Konczak et al. 2009; Gorman et al. 2020). However, a handful of small commercial orchards exist (Graham and Hart 1997; Hegarty et al. 2001; Konczak et al. 2009). Due to the intrinsic harvesting characteristics of this fruit (wild harvest), the market has periods of oversupply followed by undersupply, with demand steadily increasing in the last years (Graham and Hart 1997; Hegarty et al. 2001; Konczak et al. 2009).

The fruit of this plant is characterised by its high levels of natural ascorbic acid (vitamin C) compared with any other domesticated plants in the world (e.g. more than 75 times that of oranges) (Mditshwa et al. 2017; Williams et al. 2013, 2014, 2016). The high concentration of ascorbic acid is one of the main factors which define the price of this fruit (as fresh or dry powder) in the Australian market (ranging from AU$ 300 up to AU$ 1000 per kg).

As many other specialty foods and commodities, this food is not exempt against adulteration. Adulteration can be simply performed by the addition of either cheap chemicals or food waste products (dilution) such as non-natural sources of ascorbic acid and other chemicals (Galvin-King et al. 2018). Adulteration can cause a wide range of disruptions in the entire value chain and create fear among the consumers of the product. Thus, with the economic growth of the botanical industry, these products become targets for adulteration, which might be performed in

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order to replace prime quality products by cheaper or counterfeit materials, which may influence the health benefits of these plant materials (Galvin-King et al. 2018). Therefore, there is a high interest in the food botanical industry in detecting contamination in powders of high commercial value like KP powder (Galvin-King et al. 2018). The food industry is also highly concerned about the cross-contamination of ingredients that might increase the prevalence of food allergies or other health issues along the value chain (Galvin-King et al. 2018).

Several methods and techniques have been used to evaluate and quantify the concentration of ascorbic acid in a wide range of fruits. In particular, high-performance liquid chromatography (HPLC) is the most widely used method (Dennison et al. 1981; Campos et al. 2009; Mditshwa et al. 2017; Pereira-Netto 2018; Najwa and Azrina 2017). This method has some limitations such as high cost, need of technical skills to operate the instruments and the longer time in analysing the samples. Therefore, this method cannot be used in all the steps during the routine quality testing of foods in the industry or suppliers. Moreover, they are cumbersome, laboratory-based and not rapid for analysing a large number of samples in a short period of time and, therefore, they cannot be applied as effective screening methods. In addition, although the HPLC method can quantify the content of ascorbic acid of the samples, it cannot identify if the ascorbic acid is from an organic, inorganic and synthetic (chemical) sources (Dennison et al. 1981; Campos et al. 2009; Mditshwa et al. 2017).

Among different techniques, infrared spectroscopy (near and mid infrared) is used as a rapid, low-cost, convenient, precise, multi-analytical and non-destructive screening method for food authentication (Cozzolino 2012; Power and Cozzolino 2020). The utilization of mid infrared (MIR) technology has been widely adopted in many fresh fruits and vegetables for assessing the maturity or quality attributes of produce, measuring the abundance of bioactive compounds for different flesh types or cultivars from different geographic locations (Bureau et al. 2019). This is primarily attributed to the non-destructive nature of the technology (Cozzolino 2012, 2020; Bureau et al. 2019). The aim of this study was to investigate the ability of attenuated total reflectance (ATR) mid infrared spectroscopy in combination with classification methodologies (e.g. discriminant analysis) as a screening method to monitor and predict the level of adulteration of KP powder adulterated with synthetic ascorbic acid.

Materials and Method0073

Samples

Mature Kakadu plum (KP) fruits from different trees were harvested from the wild (Northern Territory, Australia) in 2019. The fruits were freeze dried at −50°C for 48 h (CSK Climatek, CSK Scientific, Brisbane, Australia) and ball milling (MM301 CryoMill, Retsch GmbH, Haan, Germany) to obtain a fine and homogenous powder. Vitamin C content of the freeze-dried powder samples was determined using UPLC-PDA method previously reported by Phan and collaborators (2019).

Preparation of Mixtures

The KP powder samples with high (29% DW) (namely HVITAC_KP) and low (0.3% DW) (namely LVITAC_KP) ascorbic acid content were used to prepare the mixtures. The KP powder having low ascorbic acid content was mixed together with increasing concentrations of HVITAC_KP to obtain mixtures ranging in concentrations from 1% to 25% DW (namely LH). All mixtures were homogenized by inversely mixing for 1 h on a reciprocating shaker (RP1812, Paton Scientific, Victor Harbor, SA, Australia). The concentration of vitamin C in the mixtures was monitored using UPLC-PDA.

A second set was prepared using the LVITAC_KP, and the KP powder samples was mixed with the synthetic standard ascorbic acid (>99% purity, Sigma Aldrich, NSW. Australia). A third set was used to test the limit of detection (LOD) of the method. In this case, mixtures were prepared by mixing the KP powder samples having low vitamin C content (0.25–0.3% DW) with the non-natural ascorbic acid standard. All the sample combinations were prepared in duplicate. Commercial samples of KP powder available in the market were also analysed for comparison.

Spectrum Collection and Data Analysis

Dry powder samples (KP powders, artificial mixtures and commercial powders) were scanned using a Bruker Alpha fitted with an attenuated total reflectance a platinum diamond ATR single reflection module (Bruker Optics GmbH, Ettlingen, Germany). The MIR spectra were recorded using OPUS software version 8.5 provided by Bruker Optics (Bruker Optics GmbH). Measurements were recorded in the spectral region between 4000 and 400 cm^{-1}. Each spectrum was computed using the average of 24 interferograms at a resolution of 4 cm^{-1}. Air was used as the reference background spectra. The ATR cell was cleaned with 70% (w/w) ethanol and dried with paper wipes between samples in order to minimise the carry over between samples. The spectra were exported from the OPUS software (OPUS format) to The Unscrambler X software version 11 (CAMO ASA, Oslo, Norway) for data processing and calibration development. The spectra were pre-processed using the second derivative (Savitzky-Golay algorithm 2nd polynomial order, smoothing window size of 11 points) (Savitzky and Golay 1964). The second derivative has been used in this study as it has shown to be effective at correcting for baseline effects and slope of a

spectrum (Savitzky and Golay 1964). Calibration models between the spectra (MIR) and the reference data (level of contamination) were developed using a partial least squares (PLS) regression. The optimal number of factors for the calibration model was selected based on the minimal value of the predicted residual sum of squares (PRESS) and the highest correlation coefficient (R^2) between actual and predicted values (Bureau et al. 2019). The PLS models were evaluated in terms of the number of factors, standard error of cross-validation (SECV) and correlation coefficient. The residual predictive value (RPD) was used to evaluate the accuracy of the models (Williams et al. 2017; Bureau et al. 2019; Cozzolino et al. 2019).

Results and Discussion

Figure 1 shows the MIR spectra of KP dry powder and synthetic ascorbic acid samples. The main spectral bands observed in the MIR region are associated with moisture in the range between 3700 cm^{-1} and 3100 cm^{-1} (O–H stretch) (Yang and Irudayaraj 2002; Talari et al. 2017). Two specific peaks were observed in the KP dry powder at 2915 cm^{-1} and 2815 cm^{-1}, the later absent in the synthetic ascorbic acid samples. Peaks at 1743 cm^{-1} (ester groups) and 1640 cm^{-1} (O–H bend and amide groups) overlap with protein bands at 1650 cm^{-1} (amide I), and 1450 cm^{-1} (amide II) (Yang and Irudayaraj 2002; Talari et al. 2017). The O–H bending due to a small shoulder at 1650 cm^{-1} gradually disappeared as the concentration of ascorbic acid increased in the commercial KP powder samples analysed. The intensity or absorbance around 1682 cm^{-1} (C–C) increased with an increase in the concentration of vitamin C in the sample. These bands overlap with the band at 1650 cm^{-1} (amide group) related with proteins (Yang and Irudayaraj 2002; Talari et al. 2017; Cozzolino 2020). Other bands were also evident and can be observed around 1100 cm^{-1} and around 1050 cm^{-1} associated with structural and non-structural carbohydrates (polysaccharides and sugars) (Yang and Irudayaraj 2002; Talari et al. 2017; Cozzolino 2020). In addition, two bands were observed around 775 cm^{-1} and 820 cm^{-1}. These wavenumbers were common and very similar in absorbance between the KP dry powder and synthetic ascorbic acid, and they are associated with C–H bend and C–C ring bend (Yang and Irudayaraj 2002; Talari et al. 2017).

Figure 2 a–c show the PCA score plot of the KP dry powder, artificial mixtures and commercial KP powder samples analysed using MIR spectroscopy. Figure 2 a shows the PCA score plot of all samples analysed together, mixtures and commercial samples. Figure 2 b shows the analysis of the mixtures and KP powder samples prepared in the laboratory, while Fig. 2c is the same as Fig. 2b without the inclusion of synthetic ascorbic acid. Separation between the artificial mixtures and KP powder samples (experimental and commercial samples) was observed along PC1 (72% of the variance). PC2 shows the trend of separation of KP powder samples related with the natural ascorbic acid. This can also be associated with the different origins as well as processing methods used by the producers and suppliers. Figure 2 c shows the separation in the samples associated with the non-adulterated and adulterated KP powder samples excluding the spectra of synthetic ascorbic acid for the PCA analysis. In this case, both PC1 and PC2 explained 56% and 28% of the variability. Samples were clustered together based on the level of adulteration along the PC1, while PC2 explained the changes in the MIR spectra associated with the addition mixtures of low and high KP powder samples. The PCA loadings (Fig. 3) showed that the following wavenumbers contributed to explain the main differences in the spectra of the samples (adulterated vs non-adulterated) at 1148 cm^{-1}, 1078 cm^{-1}, 987 cm^{-1}, 927 cm^{-1},

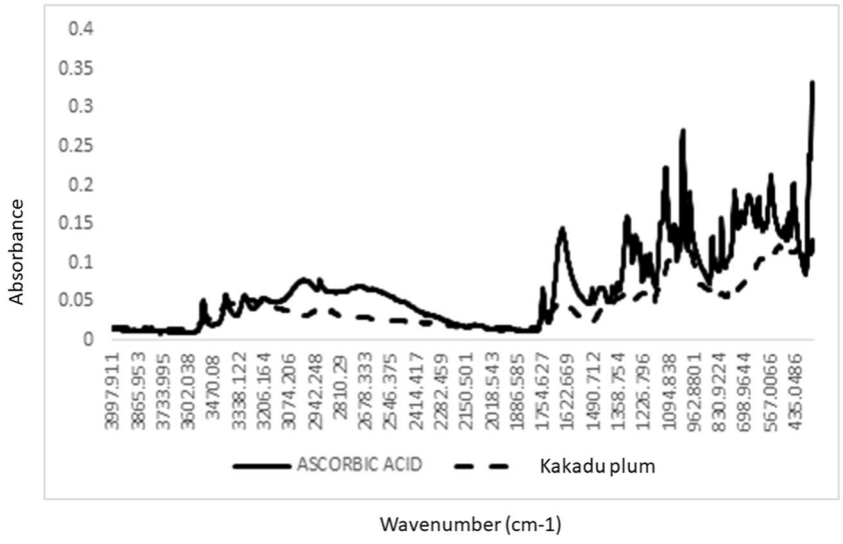

Fig. 1 Attenuated total reflectance mid infrared spectra of Kakadu plum dry powder samples and synthetic ascorbic acid

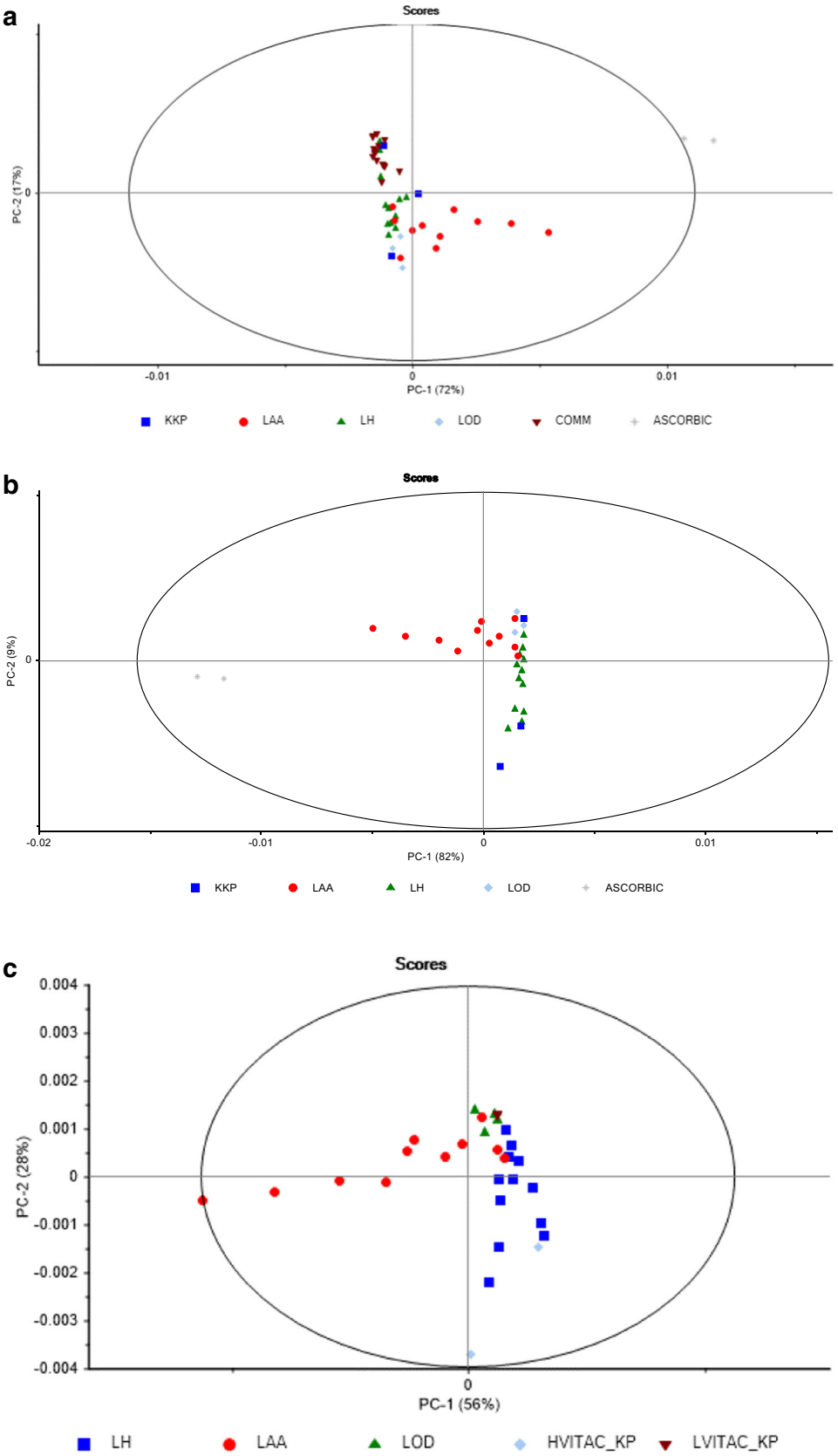

Fig. 2 Principal component score plot of Kakadu plum dry powder samples, commercial samples and powder with synthetic ascorbic acid mixtures analysed using attenuated total reflectance mid infrared spectroscopy. **a** All samples. **b** Mixture of natural and synthetic dry powder samples. **c** Mixture of natural samples dry powder samples

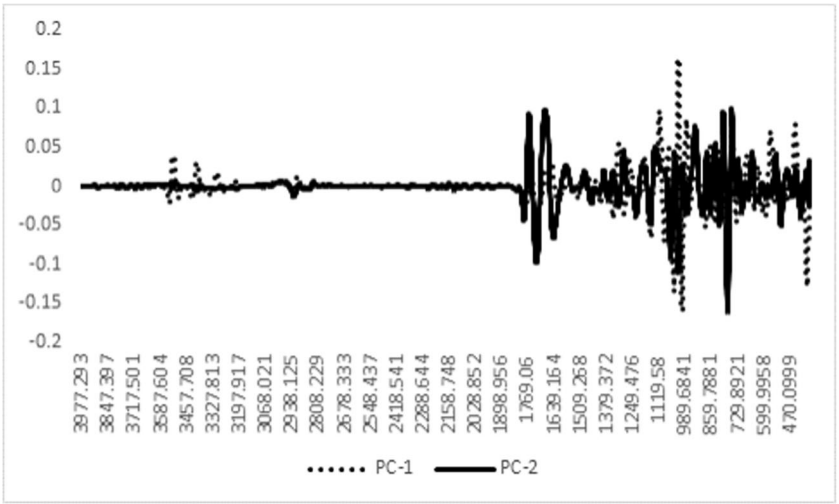

Fig. 3 Loadings derived from the principal component analysis of Kakadu plum dry powder samples and their mixtures with synthetic ascorbic acid analysed using attenuated total reflectance mid infrared spectroscopy

849 cm^{-1} and 760 cm^{-1} (Yang and Irudayaraj 2002; Talari et al. 2017). These wavenumbers are associated with protein, polysaccharides and sugars as described in the above section.

In this study, the fingerprint region was used to develop the PLS calibrations for the prediction of the level and source of adulteration in the KP dry powder samples analysed using MIR spectroscopy. The coefficient of determination (R^2) and the standard error of cross validation (SECV) obtained for the prediction of level of adulteration were 0.85 and 3.2%, respectively. The R^2 and SECV for the prediction of source of adulteration were 0.83 and 0.30%, respectively. The interpretation of the PLS loadings (after second derivative normalization) (Fig. 4) for the calibration models used to predict the level (two latent variables) and source (ten latent variables) of adulteration. The PLS loadings for the level of adulteration showed the highest loadings around 1762 cm^{-1} (esters), 1577 cm^{-1}, 1350 cm^{-1}, 1288 cm^{-1}, 1082 cm^{-1}, 1017 cm^{-1}, 938 cm^{-1}, 876 cm^{-1}, 835 cm^{-1} and 795 cm^{-1} (Yang and Irudayaraj 2002; Talari et al. 2017). These wavenumbers indicated that polysaccharides, sugars and compounds having ester groups might contribute to explain the spectral differences associated with the level of adulteration with synthetic ascorbic acid (Yang and Irudayaraj 2002; Talari et al. 2017). The highest loadings for the prediction of source of adulteration (natural vs synthetic adulteration) were observed in the range between 750 and 480 cm^{-1}. The interpretation of the PLS loadings indicated that the MIR information might be different depending on the target such as level adulteration with a synthetic source or the source of the adulteration (e.g. lower ascorbic acid with high ascorbic acid KP dry powder). Furthermore, the MIR region associated with DNA was used to develop a PLS model for the prediction of source of adulteration. The R^2 and SECV for the prediction of source using the MIR range between 800 and 400 cm^{-1} were 0.92 and 0.22%, respectively. These results indicated that MIR is capable of differentiating between natural and a synthetic source of ascorbic acid. The main PLS loadings (six latent variables) were observed around 790 cm^{-1} (Guanine in a *C3'endo/syn* conformation in the Z conformation of DNA), 602 cm^{-1} (ring deformation of phenyl groups) and 505 cm^{-1} (Talari et al. 2017). Table 1 presents the confusion matrix for the classification of adulterated and non-adulterated KP samples with synthetic ascorbic acid. Samples were 100% correctly classified as KP dry powder or synthetic ascorbic acid.

Conclusions

The results demonstrated the potential of MIR spectroscopy to predict and or monitor the level and source of adulteration in KP dry powder samples with synthetic ascorbic acid. The utilization of MIR spectroscopy showed satisfactory

Table 1 Confusion matrix for the classification of adulterated and non-adulterated Kakadu plum dry powder with synthetic ascorbic acid

	Synthetic ascorbic acid % Correct Classification	Kakadu plum dry powder % Incorrect classification
Synthetic ascorbic acid	100	0
Kakadu plum dry powder	0	100

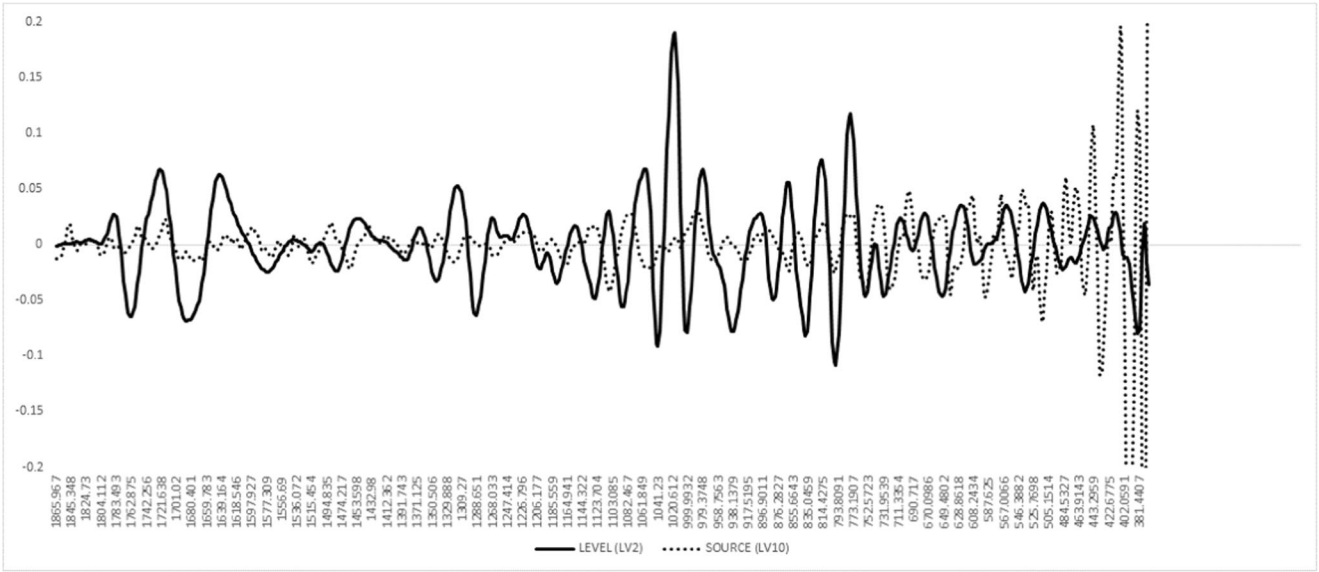

Fig. 4 Loadings derived from the partial least square regression analysis for the prediction of level of adulteration in Kakadu plum dry powder samples with synthetic ascorbic acid and their mixtures using attenuated total reflectance mid infrared spectroscopy

robustness in order to address the concerns of applying this technology to the growing KP industry in Australia. However, one of the limitations of this study is the number of samples analysed and further validation must be recommended using samples from other sources, geographic locations and harvest seasons. Despite the potential of the technology, its non-destructive characteristic, as well as the rapid and convenient measurement in routine analysis, applications have mostly been confined to research and laboratory settings. The present work may lay a practical foundation for future measurements of quality control tool in both research laboratories and the KP powder industry.

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Declarations

Ethics Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent not applicable.

Conflict of Interest Anh Dao T. Phan declares that she has no conflict of interest. Saleha Aker declares that she has no conflict of interest. Heather E. Smyth declares that she has no conflict of interest. Yasmina Sultanbawa declares that she has no conflict of interest. Daniel Cozzolino declares that he has no conflict of interest.

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Unlocking the Secrets of *Terminalia* Kernels Using Near-Infrared Spectroscopy

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Abstract

In recent years, the native food industry in Australia has increased in both value and volume due to the discovery of a wide range of phytochemicals (e.g., vitamin C, polyphenols) that have potential health benefits. Thus, plant organs and tissues of these native plants are used in a wide range of applications. In particular, the kernel of a native plum, the Kakadu plum (*Terminalia ferdinandiana*, Combretaceae) is considered to be rich in lipids and other phytochemical compounds. The aim of this study was to evaluate the use of NIR spectroscopy to analyze and characterize kernel samples and tissues of wild harvest fruit samples. The Fourier transform near-infrared reflectance spectra of cracked kernels, seeds cover tissues, and dry powder Kakadu plum kernels were acquired. Both principal component analysis and partial least squares discriminant analysis were used to analyze and interpret the spectral data. A correct classification rate of 93%, 86%, and 80% was achieved for the identification of kernel provenance using all tissues, seed coats, and the whole nuts, respectively. The results of this study reported for the first time the analysis of Kakadu plum kernels and their tissues using NIR spectroscopy.

Keywords

Near-infrared, NIR, kernel, Kakadu plum, seed coat, whole nut

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Introduction

Several native Australian plant species have been found to be relevant in applications directly associated with functional foods, nutraceuticals, and cosmetics.[1–7] In recent years, the native food industry in Australia has increased in both value and volume due to the discovery of phytochemicals and the presence of antioxidants that are directly attributed with potential health benefits.[6]

An endemic plant of economic importance in Australia is *Terminalia ferdinandiana* (Combretaceae), commonly known as Kakadu plum (KP). This tree is a semi-deciduous plant whose natural range is in the Northern Territory (Australia) and Western Australia (Kimberley region).[1–2] This plant is a key species from which native foods can be sourced, where the fruit and bark are used as ailments in folklore and Aboriginal medicine to treat different diseases.[1–7] Nevertheless, the increasing demand for functional foods and nutraceuticals has raised a lot of safety concern on the products worldwide.[1–7] Different plant parts and tissues from these native plant species are used in a wide range of applications. In particular, the kernel of KP has been reported to be rich in lipids and other phytochemical compounds (e.g., vitamin C).[8,9] Therefore, understanding the compositional characteristics and properties of KP kernel and seed tissues will be of importance to further define potential uses and applications of this plant.[8,10,11]

Currently, both portable and handheld near-infrared (NIR) instruments have been used and implemented over recent decades as effective tools to measure and monitor the quality of different agri-food products.[12,13] The well-known advantages of this technology (e.g., nondestructive, rapid and low cost, among others) make spectroscopy an

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ideal method to be used to analyze sub-products of the harvesting of KP, such as kernels, whole nut, and other seed tissues.

The aim of this study was to evaluate the use of NIR spectroscopy to analyze and characterize whole dried and milled kernel, as well as the tissues that constitute the kernel (nut and seed covers), of KP wild harvest fruit samples. The different tissues were also used to identify and trace the provenance of the kernels.

Materials and Methods

Kakadu plum (KP) fruit samples (*Terminalia ferdinandiana,* Combretaceae) were wild harvested in January 2020 from two distinctive locations in the Kimberley region of Western Australia (Australia). Kakadu plum trees ($n = 10$) from each site were randomly selected and approximately 50–100 fresh fruits were collected from each tree. Fruits samples were stored at –80 °C and thawed at room temperature (20 °C) before analysis. Samples were randomly selected from each tree for further analysis. After manually deseeding each fresh fruit, the seeds (tens seeds from each tree) were freeze dried at –50 °C, under vacuum (Lab Gear Scanvac, Brisbane, QLD, Australia). After drying, the seeds were individually cracked using an Engineers' vice size 125 (Dawn, Melbourne, VIC, Australia) to release the kernels from the seedcoats. Both the seedcoats and kernels were separately collected and kept in airtight containers at –80 °C until further analysis.

The Fourier transform near-infrared (FT-NIR) spectra of the cracked kernels (whole nut) and seed cover tissues, as well as dry powder samples (whole nut) were acquired using a Bruker Tango-R (Bruker Optics GmbH, Ettlingen, Germany), using a gold-coated integrating sphere (diffuse reflection). Samples were placed in a glass (borosilicate) cuvette with 10 mm diameter (Bruker Optics GmbH, Ettlingen, Germany). The NIR spectra were acquired using OPUS software version 8.5 provided by the instrument manufacturer (Bruker Optics, Ettlingen, Germany) using 64 interferograms and a resolution of 4 cm^{-1} in the wavenumber range of 11 550 to 3950 cm^{-1}.

Principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) were used to interpret the data.[14–16] The Unscrambler software (version 11; CAMO Analytics, Oslo, Norway) was used to pre-process the NIR data and develop the PCA and PLS-DA models. The spectra were pre-processed using Savitzky–Golay second derivative (second polynomial order, 21 smoothing points).[17] The PLS-DA was carried out to classify the samples, in this case, the different sample preparations and regions. Samples were assigned with a dummy number linked with the sample preparation utilized to the sample or region (e.g., 1 = whole nut, 2 = seed cover tissue; or 1 = region A and 2 = region B). Cross validation (leave one out) was used during PCA and PLS model development.[14–16] Samples were divided into calibration ($n = 40$) and validation ($n = 20$) sets using the Kennard–Stone algorithm [18] available in the Unscrambler software.

Results and Discussion

Cracked Seeds (Whole Nut and Seed Coats)

The second derivative NIR average spectra of whole nut and seed coat tissues are shown in Fig. 1. The average NIR spectra of the whole nut samples show prominent peaks at 8584 cm^{-1} and 8248 cm^{-1} are associated with the absorptions of the second overtone of the C–H bonds and the

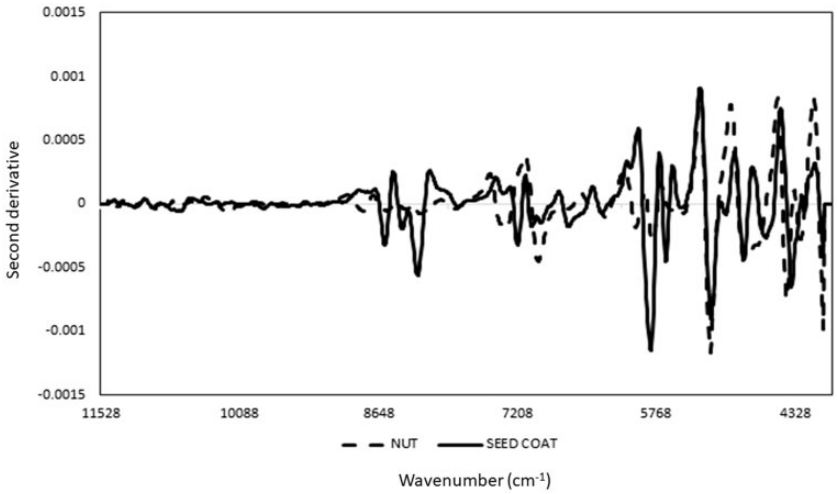

Figure 1. Second derivative of the average NIR spectra of whole nut and seed cover tissues of Kakadu plum samples analyzed using near-infrared reflectance spectroscopy.

combinations of bands of the O–H bonds.[19] At 7208 cm^{-1} and 6688 cm^{-1}, these wavenumbers are associated with either carbohydrates or the absorption of non-bonded O–H groups such as fatty acids.[19] Wavenumbers at 5824 cm^{-1}, 5672 cm^{-1}, 5208 cm^{-1}, 4864 cm^{-1}, and 4408 cm^{-1} are associated with C–H, C–C, and CH combination modes of unsaturated fatty acids related to the lipid content and fatty acid composition in the nuts.[19] These results are supported by preliminary findings by our team and reported by Akter et al.[8,9] The average NIR spectra of the seed coats show peaks at 7320 cm^{-1} (CH_3 bonds), 6984 cm^{-1} (N–H aromatic and amide groups), 5992 cm^{-1} ($CONH_2$ of peptide groups and C–H aromatic groups), 5800 cm^{-1} (CH_2 groups), 5208 cm^{-1} (C=O amide), 4840 cm^{-1} (N–H combination bands of secondary amide groups), and 4421 cm^{-1} ($CONH_2$ of peptide groups).[19] Most of these wavenumbers were associated with phenolic and aromatic compounds that are present in the seed coat.

Figure 2 shows the principal component score plot of the whole nut and seed coat samples analyzed. The first principal component explained 86%, while PC2 explained 11% of the variation in the NIR spectra. A clear separation between the types of tissue analyzed (e.g., nut versus seed coat) was observed. The PCA score plot indicated that the separation between tissues is mainly explained by PC1 (86%). The main loadings in PC1 are associated with wavenumbers at 8580 cm^{-1}, 8248 cm^{-1}, 7192 cm^{-1}, 5825 cm^{-1}, 5664 cm^{-1}, 5040 cm^{-1}, 4532 cm^{-1}, and 4368 cm^{-1} associated with C–H bonds and the combinations bands of the O–H bond, related with the presence of carbohydrates, lipids, and fatty acids.[19] Figure 2b shows the PCA score plot with the samples categorized by region. It was observed that samples tend to be clustered together according to the region along PC2 (11%). Loadings in the PC2 were associated with wavenumbers at 7008 cm^{-1}, 5842 cm^{-1}, 5656 cm^{-1}, 5208 cm^{-1}, 4880 cm^{-1}, and 4400 cm^{-1} associated with phenolic and aromatic compounds.[19] These results might indicate that aromatic compounds may be responsible for the differences between regions.

Dry and Milled Kernel Samples

The whole kernel samples were dried and milled as described in the Materials and Methods section. The second derivative NIR spectra of dry powder samples are shown in Fig. 3. The average NIR spectra of the nut show prominent absorbance bands at 10240 cm^{-1}, 8808 cm^{-1}, 8580 cm^{-1}, and 8248 cm^{-1} associated with CH_2 bonds related with lipid content.[19] Wavenumbers at 7376 cm^{-1}, 7312 cm^{-1}, 6288 cm^{-1}, 5984 cm^{-1}, 5808 cm^{-1}, 5488 cm^{-1}, 5208 cm^{-1}, 4824 cm^{-1}, 4416 cm^{-1}, and 4272 cm^{-1} were associated with C–H bonds and the combinations bands of the O–H bond, related with the presence of carbohydrates, lipids, and fatty acids as described above.[19–22]

The NIR spectra of the dry and milled combined nut and seed coat samples were analyzed by means of PCA (Fig. 4). The first and second principal components explained 75% and 17% of the variation in the NIR spectra of the samples, respectively. A separation between the provenance of the kernel analyzed using NIR spectroscopy was observed. This separation between samples is mainly explained by the first principal component (75%) where the loadings derived from PC1 showed a markedly similarity with those observed and described in the whole kernel samples (Fig. 3). Loadings explaining the separation between samples along PC2 are associated with wavenumbers at 7080 cm^{-1}, 5992 cm^{-1}, 4984 cm^{-1}, and 4504 cm^{-1}. Most of the absorbances in PC2 were associated with aromatic compounds and lipids, contributing to the observed differences between kernel provenance.

Partial Least Squares Discriminant Analysis (PLS-DA)

Table I reports the statistics for the classification of sample provenance using the three sample preparations. A correct classification rate of 93%, 86%, and 80% was achieved for kernel provenance using all tissues combined, seed coats and whole nuts, respectively. Depending on the type of tissue preparation utilized to develop the classification models, differences in the classification rates were

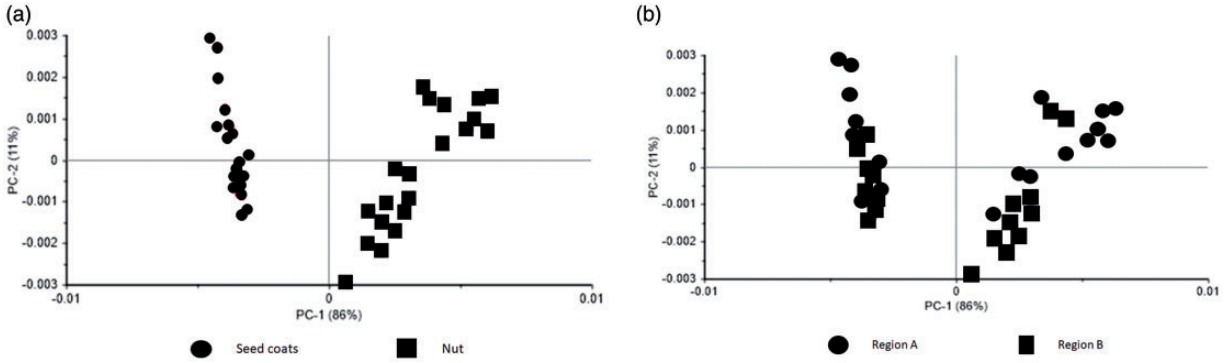

Figure 2. Principal component score plot of whole nut and seed coat Kakadu plum samples analyzed using near-infrared reflectance spectroscopy. (a) Tissue and (b) sample origin/region.

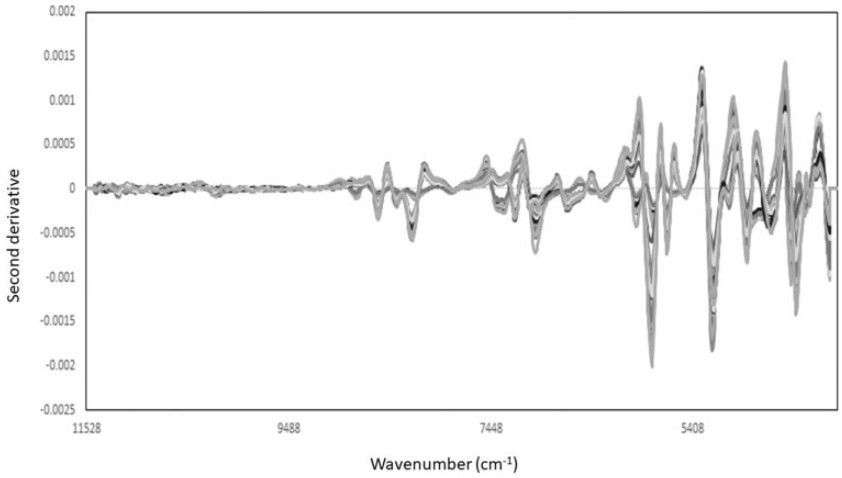

Figure 3. Second derivative NIR spectra of dry and milled whole kernel Kakadu plum samples analyzed using near-infrared reflectance spectroscopy.

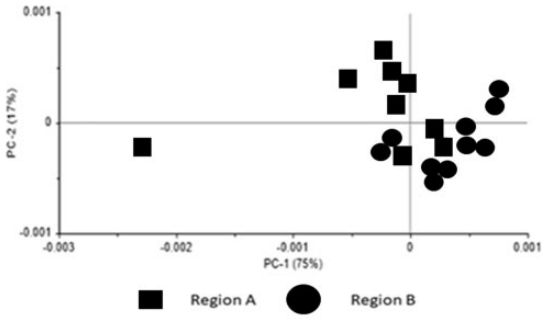

Figure 4. Principal component score plot of whole kernel (powder and milled) Kakadu samples from two different provenances analyzed using near-infrared reflectance spectroscopy.

Table 1. PLS-DA analysis classification of the provenance of Kakadu plum kernel samples according to sample preparation.

	IC%	CC%
All tissues ($n = 60$)	7% (4/60)	93% (56/60)
Seed coats ($n = 60$)	20% (12/60)	80% (48/60)
Nut ($n = 60$)	14% (8/60)	86% (52/60)

CC: correct classification; IC: incorrect classification; *n*: number of samples.

obtained. These results demonstrated that each tissue analyzed will contain different information about the origin or provenance of the sample.

Although the whole nut and the dry powder nut samples might have the same chemical composition, what has differed is the preparation between them (e.g., homogenization of the tissue). Therefore, the seed cover tissue samples that will dominate the NIR spectrum in the whole nut given that penetration is at best a few mm, will be diluted by the larger volume of nut once it is milled. In addition, the dry powder samples will have different scatter effects than the whole nut. While the study has not explored these effects, it will be important to point out that these sources of spectral variation, along with any other effects might be occurring during spectra collection.

Conclusion

The results of this study reported for the first time the analysis of Kakadu plum kernels and their tissues using NIR spectroscopy. Different classification rates for the prediction of provenance were obtained depending on the type of tissue analyzed. These results also indicated that aromatic groups and lipids might contribute in explaining the observed differences between tissues and regions. Although, these results are promising, more samples and harvest must be included in order to confirm the results obtained in this study.

Acknowledgments

The authors acknowledge the Traditional Owners of the lands on which the *Terminalia ferdinandiana* fruits were harvested and respect the knowledge and experience the Traditional Owners hold regarding the care, harvest, and use of these plants.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Regular article

Effect of sample presentation on the near infrared spectra of wild harvest Kakadu plum fruits (*Terminalia ferdinandiana*)

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ABSTRACT

Consumer awareness of the health value of native and tropical fruits and its products, is increasing, where demands for new applications are driving food industry to find novel applications of these fruits as functional ingredients. Kakadu plum fruit (*Terminalia ferdinandiana*) is endemic to Australia and has been used as a functional food or ingredient. This fruit is sold as whole fruit, puree or powder. Critical to the commercialization of this fruit is the development and use of methods and tools to better monitor and understand the quality of these products throughout the value chain. The aim of this study was to evaluate four scanning positions used to collect near infrared (NIR) spectra of Kakadu plum fruits wild harvested, in order to develop a rapid and high throughput method to monitor fruit chemical composition. A portable NIR instrument (Micro-NIR, Viavi, Milpitas, CA, USA) was used to collect the spectra of whole fruit (four positions) samples from the Kimberley region in Western Australia. The NIR data was analysed using principal component analysis (PCA) and partial least squares (PLS) regression models for moisture and total soluble solids. The results showed that the scanning position affected the performance of the models (standard error of cross validation). It was concluded that NIR spectroscopy is a promising method to assure the integrity of the value chain of these products. However, a more robust protocol must be defined when whole fruit are analysed.

1. Introduction

Several studies highlight the ability of near infrared (NIR) spectroscopy to analyse composition in fruits and vegetables [23,17,18,24,7]. In recent years, increase attention has been given to the application of miniature and portable instrumentation, this has led to the increased availability of small and precise handheld NIR spectrometers to the market [23,17,24,7,25].

The main advantages (e.g. portability, flexibility, easy to use) of this type of instrumentation provide the agri-food industries the ability to develop novel applications targeting specific steps in the food value chain [5,13,18,15,25]. In addition, these advantages make this instrumentation ideal for in field-use to monitor and measure fruit composition [5,13,15,18,20,2]. Within this context, both the fruit and vegetable industries demand easy to use instrumentation capable of carrying out real time in field analyses [5,13,15,20,2]. This will be of importance if new technology and methods are being developed to help communities to monitor the proper timing for on tree fruit ripening as well as to aid in defining the most suitable harvest dates, shelf-life and storage conditions [13,15,20,2].

Recent years have seen an increase in the information available for both the agro-food industry and consumers dealing with fruit quality and the improving of the composition standards [13,15,2,25]. Even though consumers were not always pleased about the composition and quality parameters of the different fruits available in the market due to individual preferences, storage conditions, market logistics [13,15,2], Santos et al., 2018, [25]. Determining the most suitable harvest time of tree fruits maximizes both labour efficiency and fruit quality. Therefore, the possibility to utilise handheld and portable NIR instruments directly in the field will provide the best tools to ensure optimal fruit quality [13,15,2,20,25]. In addition, these tools will help to better address the demands from consumers and the market, avoiding waste and losses [13,15,2].

Kakadu plum (*Terminalia ferdinandiana* Exell, Combretaceae) is an

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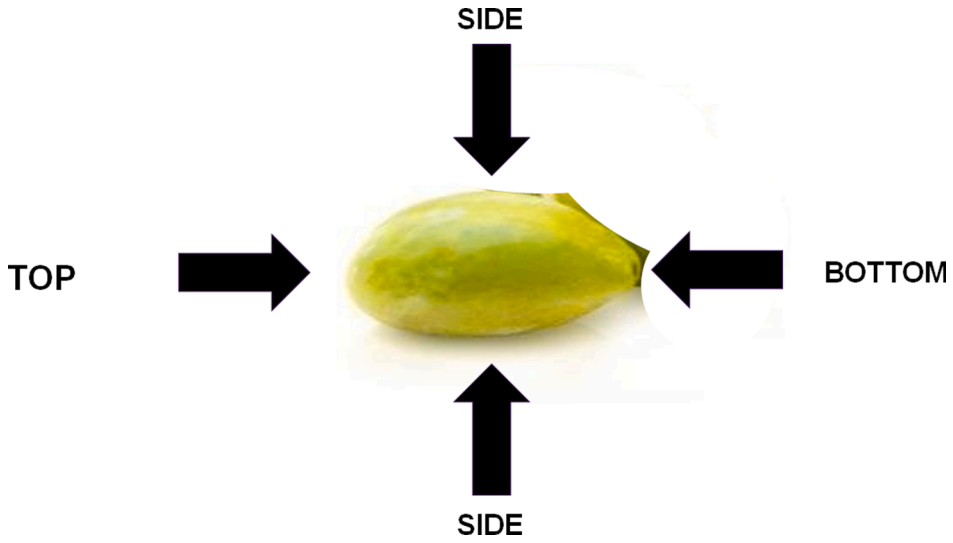

Fig. 1. Scan positions used to collect the near infrared spectra of Kakadu fruit (*Terminalia ferdinandiana*) samples.

endemic plant of Australia, with edible fruits that are extremely rich in antioxidants [8,9,10,27,26,4,11]. There are many common and Aboriginal language names for *T. ferdinandiana*. It is known as Kakadu plum, bush plum, billygoat plum, examples from Broome, Western Australia include Nyaminyari (Karajarri people south of Broome region) and Gubinge (Bardi people north of Broome). Fruits and other plant tissues of these wild botanical species have been used as a traditional medicine by the Australian Aboriginal communities [12,30,31,6,4,11]. As consumer awareness on the health benefits and properties of these fruits and derived products, increases, demands for new applications drive the food industry to find novel applications of these botanicals as a functional food ingredient [16,22,1,19,4,11]. Most of these fruits are wild harvested due to the growing and cultural practices. In this context, harvesting schedule and composition monitoring in the food value chain are very important to make the production and use of these ingredient sustainable [16,1,19,4,11].

The aim of this study was to evaluate four scanning positions used to collect NIR spectra of Kakadu plum fruits harvested in the wild in order to develop a rapid and high throughput method to monitor composition to be used in the Indigenous communities in Northern Australia.

2. Materials and methods

Kakadu plum (KP) fruit samples (*Terminalia ferdinandiana*) were wild harvested in January 2020 from two locations in Kimberley Western Australia, Australia including Nyaminyari from Karajarri country. Kakadu plum trees (n = 10) from each site were randomly selected and approximately 50–100 fresh fruits were collected from each tree. Fruits samples were stored at −80 °C and thawed at room temperature (20 °C) before analysis.

Fruit samples (n = 10) were randomly selected from each tree for further analysis. Fruit samples from each tree were blended into a puree using mortar and pestle. Total soluble solids (TSS) was determined using a digital refractometer (Hanna Instruments Ltd., Leighton Buzzard, UK)

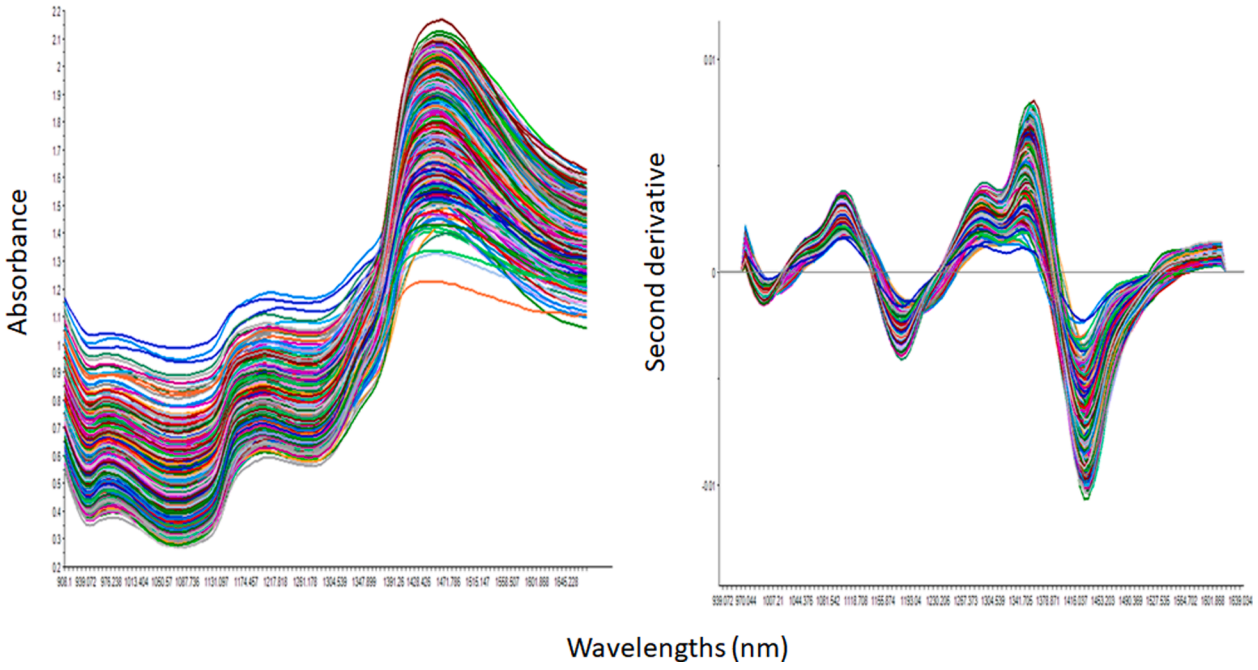

Fig. 2. Raw and second derivative of near infrared spectra of Kakadu samples (*Terminalia ferdinandiana*) fruit samples scanned at four positions.

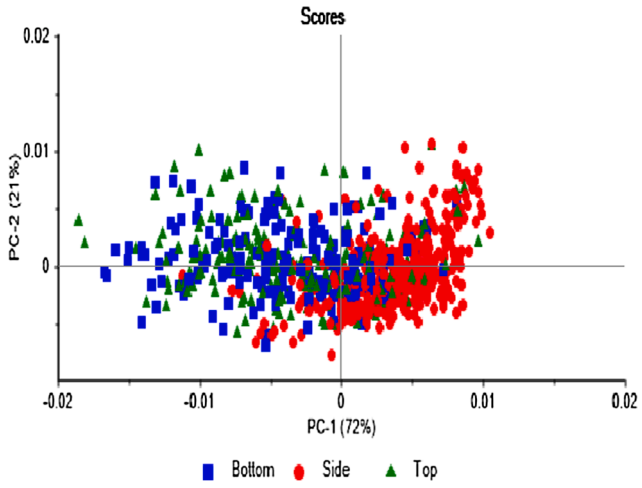

Fig. 3. Principal component score plot of Kakadu samples (*Terminalia ferdinandiana*) fruit samples scanned at four positions.

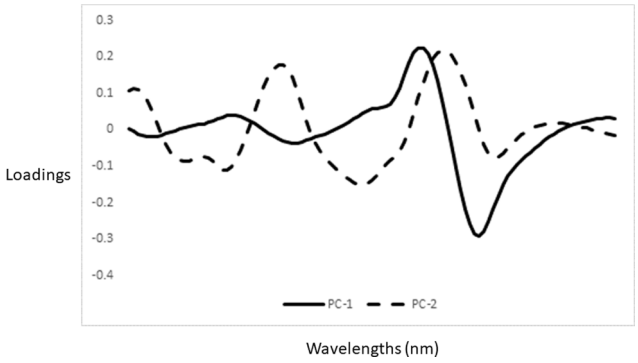

Fig. 4. Principal component loadings plot of Kakadu samples (*Terminalia ferdinandiana*) fruit samples scanned at four positions.

[27]. A Metrohm Karl Fischer pH meter (Metrohm, Herisau, Switzerland) was used for the determination of pH. Moisture (M %) in the sample was determined by the method described in William and collaborators (2014). Triplicate puree samples (3–4 g) were weighed into stainless steel dishes covered by lids and dried for approximately 16 h at 70 °C under 250 mBar pressure in a vacuum oven (Heraeus GmbH, Hanau, Germany) to a constant weight and moisture is expressed in per cent [27].

A portable NIR spectrophotometer (Micro-NIR 1700, Viavi, Milpitas, CA, USA) operating in the 950–1600 nm wavelength range, with a spectral resolution of 10 nm with no moving parts was used to collect the spectra of fruits at four positions (Fig. 1). The NIR instrument was connected through an USB interface to a notebook computer running proprietary software (MicroNIR Prov 3.1, Viavi, Milpitas, CA, USA) for the acquisition of diffuse reflectance spectra of the samples. The controlling parameters for spectral data acquisition were set at 50 ms integration time and averaging of 50 scans (MicroNIR Prov 3.1, Viavi, Milpitas, CA, USA). The reference spectra for absorbance/reflectance calculation was collected using Spectralon®. For each sample, a representative spectrum was obtained at each of the four positions.

Principal component analysis (PCA) and partial least squares (PLS) regression were used to interpret the data and to develop calibrations fro M and TSS [14,3]. The *Unscrambler* software (version 11; CAMO ASA, Oslo, Norway) was used to pre-process the data and develop the models. The pre-processing of spectral data obtained with the NIR was subjected to pre-processing in order to extract useful information from the acquired signals, because wavelength-dependent scattering effects, instrumental noise, ambient effects, and other sources of variability may affect the spectra [28,14,3]. The spectra were pre-processed using Savitzky–Golay second derivative (second polynomial order, 10th smoothing data points) [21]. PLS calibration models were developed for total soluble solids (%) and moisture (%). Cross validation (leave one out) was used during PCA and PLS model development [14,29,3].

3. Results and discussion

The raw and second derivative NIR spectra of *Terminalia ferdinandiana* fruit samples are presented in Fig. 2 (panel A and B). The raw NIR spectra showed a large scatter variability among the samples due to the different scanning positions of the fruit. The main absorbance values can be observed at 970 nm, 994 nm, 1180 nm and 1428 nm (second derivative). Absorbance values at 970 nm and 1428 nm (OH stretch first overtone) are associated with water, while the absorbance values at 994 nm and 1180 nm are associated to CH stretches (third and second overtone) mainly related with carbohydrates and other organic

Table 1
Cross validation statistics for the prediction of moisture (M) and total soluble solids (TSS) in *Terminalia ferdinandiana* fruit samples.

	N	SECV	Bias	slope	RPD	LV
ALL						
M (%)	720	0.88	−0.001	0.55	1.7	10
TSS (%)	720	0.45	0.001	0.40	1.8	10
Bottom						
M (%)	191	1.06	−0.001	0.41	1.4	6
TSS (%)	191	0.63	−0.0008	0.59	1.3	1
Side						
M (%)	380	0.90	0.001	0.58	1.6	9
TSS (%)	380	0.36	0.003	0.59	2.3	12

LV: number of optimal latent variables used to develop the models; M: moisture; N: number of samples; RPD: SD/SECV; SECV: standard error for cross validation; TSS: total soluble solids.

compounds present in the KP fruit samples analysed [32]. Three shoulders were also observed around 1100 nm, 1298 nm, 1340 nm and 1549 nm. These absorbance values might be related with CH as well, but this is more likely to represent the cellulose and hemicellulose content of the samples [32].

Fig. 3 shows the principal component score plot of the KP fruit samples scanned at four positions (refer to Fig. 1). The first two principal components explained more than 93% of the variation associated with the differences in the NIR spectra related with scanning position. It has been observed that the NIR spectra of the fruit samples scanned using the side are more "homogenous" (clustered together) compared with the top and bottom scanning positions.

Fig. 4 shows the PCA loadings derived from the PCA analysis reported in Fig. 3. Wavelengths around 970 nm, 1180 nm and 1428 nm associated with water explained most of the variation along the first principal component (PC1 72%). Loadings derived from PC2 (21%) explain the fruit-to-fruit variation. Differences in water content as well as in the distribution of water in the fruit might explain the observed differences in patterns of the PCA scores. It has been reported by other authors that water represents about 80–90% of fresh weight of any given fruit [13,15] and therefore, the water absorption bands related to the O-H bonds are predominant in fruit and vegetables NIR spectra [13,15,32]. In addition to water, other organic compounds such as carbohydrates, organic acids, proteins as well as other minor compounds that are common constituents of fruit can be observed in the NIR spectra [15,32]. This determines that the NIR spectra can be very complex exhibiting wide absorption bands as consequence of the hydrogen bonding interactions with different molecules [15,32]. It has been reported by other authors that starch and sugars may have absorption bands 920 nm associated with O-H overtones and C-H starch overtones [32]. It has been also discussed that carbohydrates such as starch and sugars might have absorptions close to those wavelengths associated with O-H and

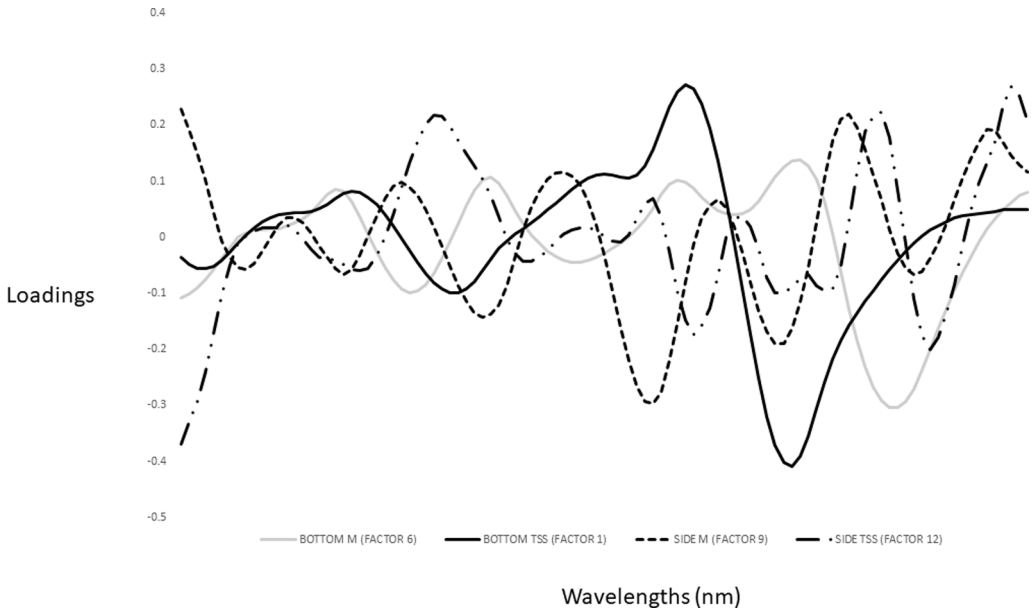

Fig. 5. Partial least squares loadings plot of Kakadu samples (*Terminalia ferdinandiana*) fruit samples scanned at four positions.

this might be very difficult to observed and interpret them in the short wavelength range [32].

In order to further evaluate the effect of the scanning position on the NIR spectra of the KP plum samples, PLS models for moisture and total soluble solids were developed using the fruit samples scanned from the bottom, side and for the combination of both (see Fig. 1). Table 1 shows the cross validation statistics for M (%) and TSS (%) for samples scanned from the bottom and side of the fruit. The standard error of cross validation (SECV) and the residual predictive deviation (RPD = SD/SECV) were used to evaluate the predictive ability of the PLS models developed to predict these parameters [14,29,3]. The SECV and RPD for M and TSS were 1.06% (RPD: 1.4) and 0.63% (RPD: 1.3) and 0.90% (RPD: 1.6) and 0.36 (RPD: 2.3) for bottom and side, respectively. Approximately, 20% and 60% variation in the SECV values between M and TSS were observed due to the different scanning position used. In all cases, the scanning of the fruit samples from the bottom returned the worst calibration statistics. Please note that the PLS calibration were not aimed to develop robust calibrations for these two parameters rather to use them as indicative of the performance related with the scanning position.

The PLS loadings **(**Fig. 5**)** were analysed and interpreted for each of the models developed. The PLS models developed for the same parameter using different scanning positions showed different loadings [14,3]. The PLS loadings for the prediction of M using the bottom scanning position utilised only two wavelengths (1174 nm and 1434 nm) while three wavelengths were used for the development of models for TSS (1143 nm, 1273 nm, and 1513 nm). The number of wavelengths contributing to the PLS loadings in the models using fruit samples scanned on the side increased to four (1199 nm, 1332 nm, 1420 nm and 1527 nm) and five (1100 nm, 1230 nm, 1428 nm, 1465 nm, 1569 nm) for the prediction of M and TSS, respectively [32]. The observed differences in the number of wavelengths used by each of the models might indicate an effect of the scanning position on the information collected by the NIR instrument. This is very important, as the NIR spectra collection protocol will be developed to scan and analyse fruit samples in the wild.

4. Conclusions

The results of this study showed the effect of scanning position on the NIR cross validation statistics for moisture and total soluble solids. The PLS loadings for the M and TSS models varies depending on the scanning position used to collect the NIR spectra. These results are very relevant and of great value if protocols based on the use of NIR spectroscopy are to be used to schedule harvest in wild fruit.

Funding

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Declaration of Competing Interest

None.

Acknowledgments

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An Infrared Analysis of *Terminalia ferdinandiana* Exell [Combretaceae] Fruit and Leaves—Towards the Development of Biospectroscopy Tools to Characterise Uniquely Australian Foods

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Abstract

Knowledge about the inherent complexity of the composition, nutrition, and physiology of different plant tissues and parts is required as this will allow for the discovery of new or improved properties (e.g. new bioactive compounds with health-benefitting activities, antimicrobial substances to reduce food spoilage activity). This paper reports the use, analysis and interpretation of the mid-infrared spectra of different plant parts (fruits and leaves) from an Australian native tropical plant, *Terminalia ferdinandiana* Exell [Combretaceae]. The use of infrared spectroscopy together with chemometric techniques will allow to obtain different levels of information about the chemical composition of leaf and fruit samples associated with maturity. Freeze-dried powder and puree of *Terminalia ferdinandiana* is commercially available as a functional food ingredient, whereas leaves or any other tissues are not considered as functional ingredients. The use of mid-infrared spectroscopy can provide an initial screening tool for the discovery and development of new ingredients and products. This technology can be an easy to use, cost-effective and high throughout method to ensure quality and authenticity of food products throughout the value chain. The incorporation of these techniques might be considered the base of bio spectroscopy analysis as proxies to study tropical species with distinctive bioactive properties and nutritional value such as *Terminalia ferdinandiana*.

Keywords Infrared · High throughput · Maturity · *Terminalia ferdinandiana* · Kakadu plum · Bioactivity

Introduction

Recent developments in disciplines intimately related with fruit production systems (e.g. plant breeding, physiology, biochemistry, chemistry, genetics and nutrition) allowed for a better understanding of the composition, health benefits, and nutritional quality of these plant-based products (Dembitsky et al. 2011; Moure et al. 2001; Schieber et al. 2001; Guaadaoui et al. 2014; Kaur and Kapoor 2001; Ignat et al. 2011; Cozzolino 2009, 2011; Türker-Kaya and Huck 2017). Knowledge about the inherent complexity and relationship of fruit composition, nutrition, and plant physiology are required as these will allow for the discovery of new or improved properties such as the discovery of new bioactive compounds with health-benefitting activities and the development of antimicrobial substances to reduce food spoilage, among others (Dembitsky et al. 2011; Moure et al. 2001; Schieber et al. 2001; Guaadaoui et al. 2014; Kaur and Kapoor 2001; Ignat et al. 2011; Cozzolino 2009, 2011, 2015; Türker-Kaya and Huck 2017). However, these new challenges will require moving towards to a more holistic, interdisciplinary and systematic approaches (Dembitsky et al. 2011; Moure et al. 2001; Schieber et al. 2001; Guaadaoui et al. 2014; Kaur and Kapoor 2001; Ignat et al. 2011; Cozzolino 2009, 2011).

It has been demonstrated the main advantages of incorporating data analysis methods (e.g. chemometrics) to modern analytical instrumental techniques to those based in vibrational spectroscopy near infrared (NIR), mid-infrared (MIR) and Raman spectroscopy (Cozzolino 2009, 2011; Baranska and Schulz 2006; Baranska et al. 2004; Türker-Kaya and Huck 2017). In particular, the combination of mathematics and

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vibrational spectroscopy allowed for the identification, monitoring and quantification of the compositional and nutritional value of fruits and vegetables (Cozzolino 2009, 2011, 2015; Baranska and Schulz 2006; Baranska et al. 2004).]

The compositional characteristics, health and nutritional properties of fruits are of increasing importance to the consumers (Dembitsky et al. 2011; Moure et al. 2001; Schieber et al. 2001; Guaadaoui et al. 2014; Kaur and Kapoor 2001; Ignat et al. 2011). It is well recognised that macro-nutrients (e.g. carbohydrates, proteins, and lipids) and micro-nutrients (e.g. vitamins and macro and micro-minerals) are essential for maintaining the health and wellbeing of humans (Dembitsky et al. 2011; Moure et al. 2001; Schieber et al. 2001; Guaadaoui et al. 2014; Kaur and Kapoor 2001; Ignat et al. 2011; Cozzolino 2009, 2011). Besides these essential nutrients, other compounds, the so-called secondary plant metabolites or phytochemicals, are abundant in plants and plant-derived products (Dembitsky et al. 2011; Moure et al. 2001; Schieber et al. 2001; Guaadaoui et al. 2014; Kaur and Kapoor 2001; Ignat et al. 2011; Cozzolino 2009, 2011). A diverse range of health benefits is attributed to these phytochemicals, which can be subdivided in polyphenols, carotenoids, allyl-sulphides, isothiocyanates, phytosterols and other bioactive compounds (Dembitsky et al. 2011; Moure et al. 2001; Schieber et al. 2001; Guaadaoui et al. 2014; Kaur and Kapoor 2001; Ignat et al. 2011; Cozzolino 2009, 2011).

In addition, the matrix concentration and availability of these compounds is influenced by the environment (e.g. soil quality, rainfall, temperature, UV-radiation, etc.), genetics and pre- and post-harvest treatment of the plants (Cozzolino 2009, 2011; Baranska and Schulz 2006; Baranska et al. 2004). Therefore, the characterisation and measurement of these compounds is of importance in food science and technology (Cozzolino 2009, 2011; Baranska and Schulz 2006; Baranska et al. 2004). Modern instrumental methods such as chromatography [e.g. gas chromatography, ultrahigh performance liquid chromatography (UHPLC)], mass spectrometry (MS), electrophoresis, NMR spectroscopy and vibrational spectroscopy techniques allow to handle a great number of samples and measure a broad range of chemically different analytes (Cozzolino 2009, 2011; Baranska and Schulz 2006; Baranska et al. 2004).

One of the recognised advantages of vibrational spectroscopy (e.g. NIR, MIR) in modern food analysis is its capability to record the so-called *fingerprinting* of a given sample or even a compound (McGoverin et al. 2010; Karoui et al. 2010; Lam et al. 2005; Khatib et al. 2017; Oliveira-Folador et al. 2018; Song et al. 2018; Saad et al. 2017; Tamburini et al. 2017; Deak et al. 2015; Rungpichayapichet et al. 2015). The incorporation of vibrational spectroscopy methods alone or in combination with other instrumental analytical techniques is allowed for the development and implementation of high-throughput methods, minimising the existing requirements for sample preparation and processing (McGoverin et al. 2010; Karoui et al. 2010; Lam et al. 2005; Khatib et al. 2017; Oliveira-Folador et al. 2018; Song et al. 2018; Saad et al. 2017; Tamburini et al. 2017; Deak et al. 2015; Rungpichayapichet et al. 2015). Therefore, the use of vibrational spectroscopy, in particular MIR, has been extended to several applications in fruits and medicinal plants where the prediction of antioxidants (e.g. phenolic compounds), post-harvest monitoring of fruits, and measure of bioactive compounds are few examples of this application (Oliveira-Folador et al. 2018; Song et al. 2018; Saad et al. 2017; Tamburini et al. 2017; Deak et al. 2015; Rungpichayapichet et al. 2015; Arendse et al. 2018; Tilahun et al. 2018; Dong et al. 2013; Zheng et al. 2017).

This paper reports the use, analysis and interpretation of the mid-infrared spectra of different plant parts/tissues (leaves and fruits) sourced from an important native Australian tropical plant, *Terminalia ferdinandiana* Exell [Combretaceae], commonly known as Kakadu plum as proxy to evaluate the antioxidant capacity of this tropical plant.

Materials and Methods

Field Work and Samples

Fieldwork for this study was conducted in undeveloped bushland in the north-western corner of the Charles Darwin University, Casuarina campus, in the city of Darwin (NT, Australia) within 1 km of the coastal shoreline (Woods 1995). This region is representative of *Terminalia ferdinandiana* Exell [Combretaceae] environment. Leaves were collected between November 2017 and June 2018. A total of 15 different maturity stages from 10 individual trees were collected. For the purpose of this study, five maturity stages (e.g. 2, 3, 6, 10, 15) from 3 individual trees were randomly selected for analysis due to time constraints. Leaves at maturity stages 2 and 3 represented immature leaves, stages 6 and 10 represented mature leaves and stage 15 contained the senescing leaves.

T. ferdinandiana fruits were collected in May 2019 from Delye outstation, NT, from two specified individual trees. From each tree, collected fruits were divided into 4 stages of maturity depending on fullness of the fruits as per the maturity indices provide in the 2018 AgriFutures report (Sultanbawa et al. 2018). Fruit samples at maturity stage 1 represented the most immature fruits, while stage 4 represented the most mature fruits (Sultanbawa et al. 2018).

All the collected plant material were stored in air tight, sealable plastic bags and transported fresh, under refrigeration to Brisbane and stored at -20 °C until use. Prior to analysis, both leaf and fruit samples were subjected to freeze-drying (GAMMA 1-15LSC, Christ, Linz, Austria) and finely ground

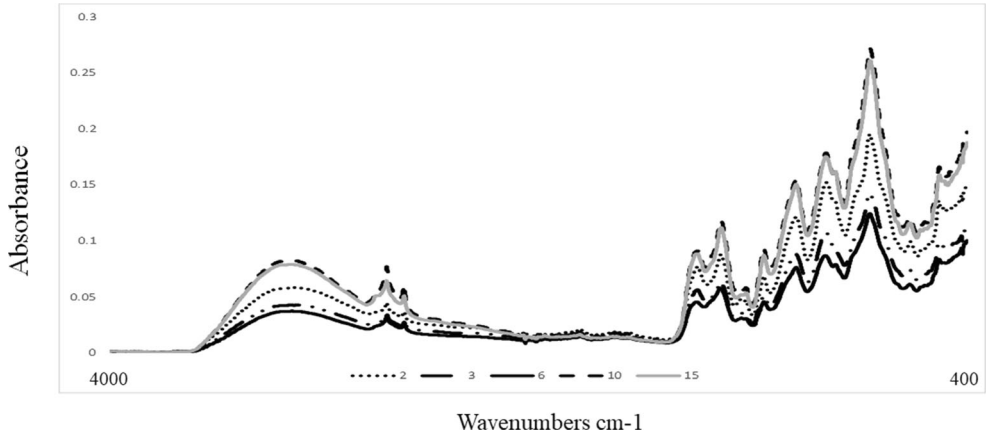

Fig. 1 Mid-infrared spectra of *T. ferdinandiana* leaf samples collected at different maturities

to homogeneous powder in a Retsch MM400 ball mill (Retsch GmbH, Haan, Germany) at a speed of 30 Hz for 1 min.

Spectra Collection

Freeze-dried powder samples (~ 0.1 g dw) of fruit and leaves were analysed using a PerkinElmer MIR *Spectrum 100*™ (400 – 4000 cm^{-1}) instrument equipped with a universal ATR Accessory with a diamond crystal (PerkinElmer, Inc.; Waltham, MA, USA, www.perkinelmer.com). Data were collected by using PerkinElmer Spectrum 6 software (PerkinElmer, Inc.). For each sample, 32 scans were obtained and co-added for each sample at a resolution of 4 cm^{-1}. A background spectrum (air) was obtained by collecting 32 scans following cleaning of the crystal with a mixture of ethanol and water.

Data Analysis

Spectra were exported in csv format into The Unscrambler (Camo, Norway) software for chemometric analysis. Principal component analysis (PCA) was carried using cross-validation (one leave out) (Cozzolino et al. 2019). Before PCA analysis, the spectra were pre-processed using Savistky-Golay second derivative (2nd polynomial order and 10 data points smoothing) (Savitzky and Golay 1964).

Results and Discussion

Spectra Interpretation

The average MIR spectra of leaf samples collected from different trees and maturity stages are shown in Fig. 1. It can be observed that the absorbance at different frequencies increases as maturity advances. The MIR peaks at 2960 cm^{-1}, 2919 cm^{-1}, and 2850 cm^{-1} are associated with asymmetric and symmetric C–H stretching of CH_2 (Bureau et al. 2009, 2012; Trevisan et al. 2012; Giovanelli et al. 2014; Ruiz et al. 2008). In the fingerprint region, peaks that are changing with the advance of maturity were observed around at 1711 cm^{-1}, 1615 cm^{-1}, 1539 cm^{-1}, 1514 cm^{-1}, 1448 cm^{-1}, 1321 cm^{-1}, 1206 cm^{-1}, 1162 cm^{-1}, 1031 cm^{-1}, 870 and 761 cm^{-1}. In the MIR fingerprint region absorbance values at 1711 cm^{-1}, 1615 cm^{-1}, 1539 cm^{-1}, 1514 cm^{-1} are associated with amide I and II bands (Bureau et al. 2009, 2012; Trevisan et al. 2012; Giovanelli et al. 2014; Ruiz et al. 2008). These frequencies indicated the changes in compounds containing nitrogen (e.g. proteins, amino acids) (Bureau et al. 2009, 2012; Trevisan et al. 2012;

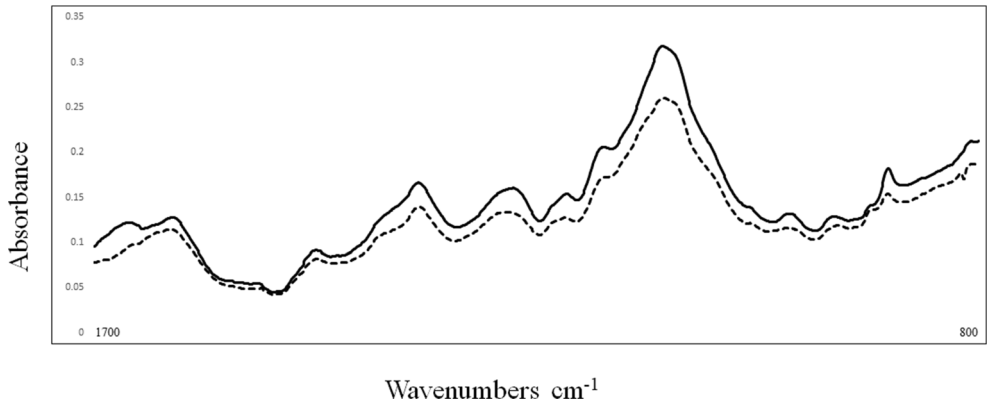

Fig. 2 Mid-infrared spectra of *T. ferdinandiana* berry samples collected at two different maturities

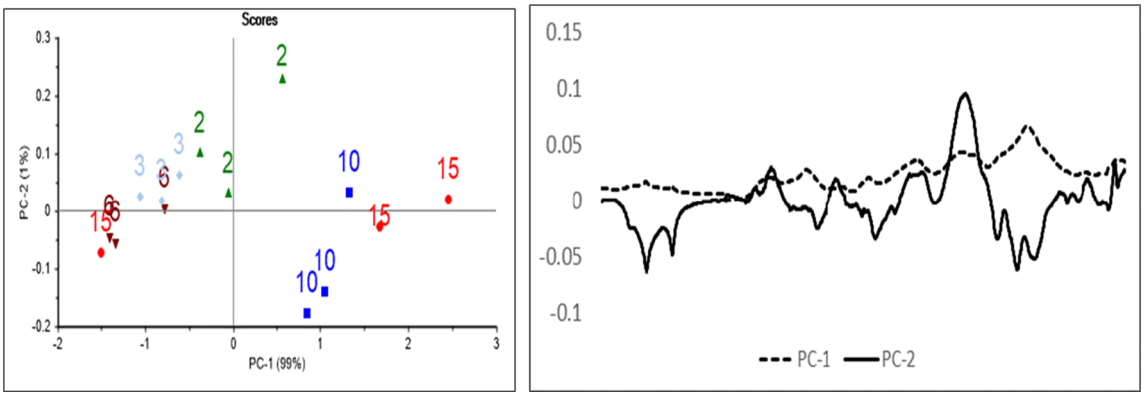

Fig. 3 Principal component score plot and loadings of *T. ferdinan*diana leaf samples analysed using mid-infrared spectroscopy

Giovanelli et al. 2014; Ruiz et al. 2008). In addition, absorbance or peaks around 1615 cm^{-1} might be associated with the C═O stretching of flavonoids, whereas the peak around 1711 cm^{-1} is associated with carbonyl stretching of carboxylic acids. These frequencies or peaks at 1162 cm^{-1} and 1031 cm^{-1} can be associated with polysaccharide rings (e.g. starch) (Bureau et al. 2009, 2012; Trevisan et al. 2012; Giovanelli et al. 2014; Ruiz et al. 2008).

The average MIR spectra of fruit samples analysed at two maturities are shown in Fig. 2. It can be observed that the absorbance values at different frequencies increase as maturity advances. Absorbance at 2960 cm^{-1}, 2919 cm^{-1}, and 2850 cm^{-1} frequencies is also associated with asymmetric and symmetric C–H stretching of CH_2 groups (Bureau et al. 2009, 2012; Trevisan et al. 2012; Giovanelli et al. 2014; Ruiz et al. 2008). Both organic acids and sugars have been reported to have absorbance values in the MIR range between 1500 and 1200 cm^{-1} (Bureau et al. 2009, 2012; Trevisan et al. 2012; Giovanelli et al. 2014; Ruiz et al. 2008). Peaks between 1200 and 900 cm^{-1} are associated with the characteristic absorptions of stretching of C–O bonds (e.g. alcohol moieties) associated with pectins, cellulose and hemicellulose in fruit of different species (Kacurakova et al. 2000; Heredia-Guerrero et al. 2014). Peaks at 1142 cm^{-1}, 1101 cm^{-1} and 1030 cm^{-1} corresponding with C–C, C–O stretching vibration and C–O-H bending vibrations are associated with saccharides and glycosides (Bureau et al. 2009, 2012; Trevisan et al. 2012; Giovanelli et al. 2014; Ruiz et al. 2008; Heredia-Guerrero et al. 2014). Several authors have also reported that the region between 1500 cm^{-1} and 900 cm^{-1} is characterised by the presence of bands derived from aromatic and flavonoid groups (e.g. aromatic ring stretch of the phenol O-H bend, the aromatic C–H in-plane bend and the C-O stretch of phenolic compounds) (Fernandez and Agosin 2007). A small peak around 924 cm^{-1} was reported to be associated with carotenoids in tomato fruits or with the sugar structure (Skolik et al. 2019). The peaks around 1215 and 1230 cm^{-1} might be associated with flavonoid-based tannins (Fernandez and Agosin 2007; Skolik et al. 2019). This peak was reported to be associated with the ethereal C–O asymmetric stretching vibration arising from the pyran-derived ring structure of this class of compounds (Bureau et al. 2009, 2012; Trevisan et al. 2012; Giovanelli et al. 2014; Ruiz et al. 2008). In addition, the peak around 877 cm^{-1} might be associated with either vibrations of minerals or C–H aromatic and N–H amines (Bureau et al. 2009, 2012; Trevisan et al. 2012; Giovanelli et al. 2014; Ruiz et al. 2008).

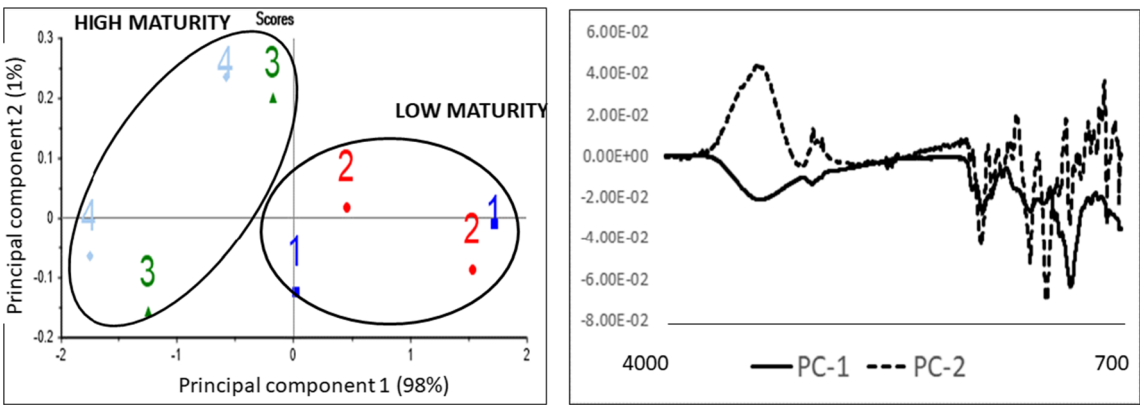

Fig. 4 Principal component score plot and loadings of *T. ferdinandiana* fruit samples analysed using mid-infrared spectroscopy

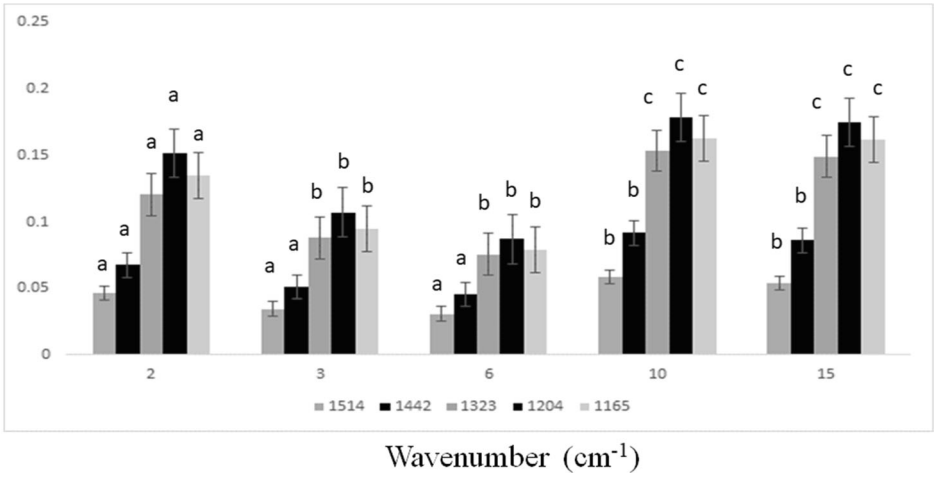

Fig. 5 Absorbance values at specific wavenumbers derived from the analysis of *T. ferdinandiana* leaf samples using mid-infrared spectroscopy. Means with the same letter are not significantly different ($P \leq 0.05$).

Principal Component Analysis

Figures 3 and 4 show the PCA score plot and loadings for the leaf and fruit samples, respectively. The PCA score plot demonstrates a clear separation between samples along PC1 (99% explanation) attributed to the variation between young and mature leaves. The majority of the variance was captured in the first PC, while only 1% was explained by PC2. This might be explained by the variation within the mature leaves and within the young leaf samples associated with their chemical composition. Similarly, the PCA score plot of the fruit samples shows a clear separation between fruit samples collected at different maturity stages (first PC 98%, while only 1% was explained by PC2). Loadings derived from both PCA analyses resemble the same frequencies as described in Figs. 1 and 2 for both leaf and fruit samples, respectively.

Analysis of Absorbance at Specific Wavenumbers

Another way to analyse and interpret the MIR data is to plot the absorbance values at specific wavenumbers to observe any changes related with physiology and maturity of the plant parts. Absorbance values at selected wavenumbers in both leaf and fruit samples are shown in Figs. 5 and 6, respectively. The absorbance values of three replicates were analysed statistically (Student *t*test) ($p < 0.05$) where the bars represent the standard error. It was observed that the absorbance values at 1514 cm^{-1} slightly increase as the maturity advances associated with aromatic and nitrogenous compounds. However, frequencies at 1442 cm^{-1}, 1332 cm^{-1}, 1004 cm^{-1} and 1165 cm^{-1} decrease up to half maturity and then start to increase until full maturity (Bureau et al. 2009 and 2012; Trevisan et al. 2012; Giovanelli et al. 2014; Ruiz et al. 2008; Skolik et al. 2019). In the fruit, an increase in the absorbance values in all the frequencies as the advance of maturity was observed. The increase in absorbance indicated the change in biochemical compounds and composition in the fruit such as cutin, phenolic compounds, polysaccharides, waxes, and volatile organic chemicals (Bureau et al. 2009 and 2012; Trevisan et al. 2012; Giovanelli et al. 2014; Ruiz et al. 2008; Skolik et al. 2019). Of special interest are the absorbance values around 1200 cm^{-1} that might be associated with monoterpenes and characteristic volatile organic compounds present at ripe stages of fruits (Bureau et al. 2009, 2012; Trevisan et al. 2012; Giovanelli et al. 2014; Ruiz et al.

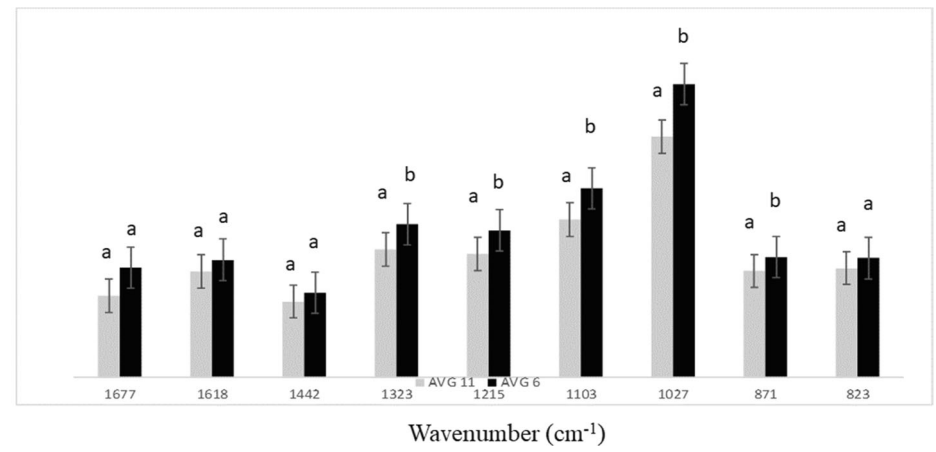

Fig. 6 Absorbance values at specific wavenumbers derived from the analysis of *T. ferdinandiana* fruit samples using mid-infrared spectroscopy. Means with the same letter are not significantly different ($P \leq 0.05$)

2008; Skolik et al. 2019). This frequency might represent a distinctive peak of the fingerprint spectrum of ripe fruit (Skolik et al. 2019). Similar trends were previously reported by other authors (Bureau et al. 2009, 2012; Trevisan et al. 2012; Giovanelli et al. 2014; Ruiz et al. 2008; Skolik et al. 2019). The same trend can be observed to changes in compounds containing aromatic groups such as phenolic compounds (Fernandez and Agosin 2007).

The number of research reports on the use of ATR-MIR spectroscopy to study the physiology, chemical and functional properties of tropical fruits has been limited. The characterization of spectral features of the *T. ferdinandiana* plant provides a first but crucial step in understanding the composition and nutritional value of this important indigenous plant. Furthermore, these data will also contribute to the development and sustainable production of novel *T. ferdinandiana* food products with functional characteristics and properties.

Conclusions

The use of MIR spectroscopy together with different data analytical tools (e.g. chemometrics, single peak analysis) allowed to obtain different levels of information about the chemical composition of different plant parts from *T. ferdinandiana* such as leaves and fruits. The information recorded in the MIR spectra can be associated with different bioactive compounds as well as other molecules of nutritional importance. The use of MIR spectroscopy provides an initial screening tool for the discovery and development of new ingredients and products. These techniques are now considered the base of biospectroscopy analysis.

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Compliance with ethical standards

Conflict of Interest Author Yasmina Sultanbawa declares that she has no conflict of interest. Author Mridusmita Chaliha declares that she has no conflict of interest. Author Anh Dao T. Phan declares that she has no conflict of interest. Author Sandra M. Olarte Mantilla declares that she has no conflict of interest. Author Gaby Netzel declares that she has no conflict of interest. Author Michael E. Netzel declares that he has no conflict of interest. Author Heather Smyth declares that she has no conflict of interest. Author Daniel Cozzolino declares that he has no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Informed consent not applicable.

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The use of vibrational spectroscopy to predict vitamin C in Kakadu plum powders (*Terminalia ferdinandiana* Exell, Combretaceae)

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Abstract

BACKGROUND: The objective of this study was to evaluate the feasibility of using either mid-infrared (MIR) or near-infrared (NIR) spectroscopy to predict the vitamin C content in Kakadu plum (*Terminalia ferdinandiana* Exell, Combretaceae) powder samples. Vitamin C is the main and quality-determining bioactive compound in Kakadu plum (KP). Kakadu plum powder samples were analyzed by ultra-performance liquid chromatography coupled to a photodiode array detector (UPLC-PDA) and scanned using both MIR and NIR spectroscopy.

RESULTS: The coefficient of determination (R^2) and the standard error in cross validation (SECV) for vitamin C were 0.93 and 1811 mg 100 g dry weight (DW) and 0.91 and 1839 mg 100 g DW using MIR and NIR spectroscopy, respectively. The coefficient of correlation and the standard error of prediction (SEP) obtained using the independent set (n = 5) were 0.65 (SEP: 2367 mg 100 g DW) and 0.73 (SEP: 4773 mg 100 g DW) using MIR and NIR spectroscopy, respectively.

CONCLUSIONS: The results obtained in this study clearly showed that it is possible to calibrate IR spectroscopic instruments for the measurement of vitamin C in KP plum powder samples. Mid-infrared spectroscopy showed the most promising results; however, Fourier transform near-infrared (FTNIR) spectroscopy also produced models capable of good quantification of this important bioactive compound and vitamin. These findings are promising in terms of using high-throughput IR spectroscopy as a routine technology to determine vitamin C in plant-based foods and derived products.

Keywords: vitamin C; NIR; MIR; Kakadu plum

INTRODUCTION

Ascorbic acid or vitamin C is one of the water-soluble vitamins.[1,2] Vitamin C is the generic term for all compounds exhibiting the biological activity of L-ascorbic acid, which is the main biologically active form of the vitamin.[1,2] This vitamin is widely distributed in raw fruits, vegetables, and other food products such as fruit juices and sport drinks.[2-4] Vitamin C is not only an essential nutrient, but is also an important natural antioxidant.[2] The recommended dietary intake (RDI) of vitamin C in Australia is 45 mg day^{-1} for adults.[3-4] Citrus fruits are well known dietary sources of natural vitamin C ranging from 16.8 to 43.6 mg 100 g fresh weight (FW).[5] Tropical fruits such Acerola (*Malpighia emarginata*) and Camu-camu (*Myrciaria dubia*) are reported to have high levels of vitamin C – up to 1677 mg 100 g FW and 2280 mg 100 g FW, respectively.[6] Several studies have also reported on the very high levels of vitamin C in Kakadu plum tissues ranging from 2700 up to 5300 mg 100 g FW.[7-13] In addition to their health benefits, processing and storage of fruits and vegetables (e.g. heating) can cause significant losses of vitamin C, resulting in lowering the quality of this ingredient and end products.[7-13]

There are several techniques available to determine vitamin C and these include colorimetric, titrimetric, ultraviolet (UV) spectrophotometric, chemiluminescence, kinetic, electrochemical, and chromatographic methods.[14,15] These methods have their advantages and disadvantages – for example, they might require large quantities of sample, or be unsuitable for the analysis of colored samples (e.g. titrimetric) – which limits their use in the food value chain.[14-17] On the other hand, they are highly accurate but time consuming and cost-intensive like (ultra-)high-performance liquid chromatography ((U)HPLC).[14-17] There is thus a need to develop rapid and cost-efficient high-throughput methods to quantify vitamin C in food products for quality control purposes and to monitor its stability in the value chain. In this context, the use of vibrational spectroscopy to measure and monitor dietary and bioactive compounds in natural

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food ingredients has not been fully investigated and few reports can be found in the scientific literature.[18]

Kakadu plum (KP) (*Terminalia ferdinandiana* Exell, Combretaceae) is an endemic plant of Australia, with edible fruits that are extremely rich in antioxidant compounds.[8-13] Kakadu plum plant has a long history of being used for its food and medicinal properties by the Aboriginal people in Australia.[19-21] The fruits and other tissues of this plant have been used to cure headaches, to alleviate symptoms of colds and flu or as an antiseptic.[8-13] Several studies have reported the composition and distribution of the bioactive compounds including vitamin C in different tissues of KP and derived products.[8-22] The fruit of this plant contains high concentration of ascorbic acid (3 to 5.5% FW) and other antioxidants and bioactive compounds such as phenolic compounds (e.g. ellagic acid) when compared with other commercial fruits such as strawberries (*Fragaria* × *ananassa*.), *Rubus* berries including raspberries (*Rubus idaeus*), and boysenberries (*Rubus ursinus* × *idaeus*), or muscadine grapes.[7-13]

The objective of this study was to evaluate the feasibility of using either mid-infrared (MIR) or near-infrared (NIR) spectroscopy to predict the vitamin C content in KP powder samples from different sources.

MATERIALS AND METHODS

Samples

The KP powder samples (n = 80) for analysis were obtained from a wide range of sources, including commercial powder samples (n = 29) purchased from suppliers in Australia, freeze-dried powder samples (n = 23) obtained from fresh samples from wild harvested fruit in 2019 at different maturity stages from the Northern Territory (Australia), and freeze-dried and oven-dried powder samples (n = 28) obtained from a commercial KP puree purchased from the Traditional Homeland Enterprises Holding Co Pty Ltd (Morwell, VIC, Australia). Five fresh KP powder samples obtained in 2020 and sourced from a commercial supplier were used as validation set.

Vitamin C analysis

Vitamin C content was measured using an ultra-performance liquid chromatography coupled to a photodiode array detector (UPLC-PDA) as previously reported by Campos and co-workers with slight modifications.[23] Briefly, a 100 mg KP powder sample was extracted with 3% meta-phosphoric acid (*w/v*) containing 8% acetic acid (*v/v*) and 1 mmol L^{-1} Ethylenediaminetetraacetic acid (EDTA) on a reciprocating shaker (RP1812, Paton Scientific, Adelaide, SA, Australia) for 15 min in the dark at room temperature. Ultra-sonication was subsequently applied to the samples for 15 min, followed by centrifugation at 3900 rpm for 5 min (Eppendorf Centrifuge 5825 g, Eppendorf, Hamburg, Germany). Supernatants were retained, whilst the residues were re-extracted twice with the procedure described above. The supernatants were combined and the reduction of dehydroascorbic acid (DHAA), which was also present in the extracts/samples, to L-ascorbic acid (L-AA) was performed following the method of Spinola and co-workers,[17] prior to UPLC-PDA analysis. Total vitamin C (L-AA + DHAA) was determined using a Waters Acquity™ UPLC-PDA System (Waters, Milford, MA, USA) and a Waters HSS-T3 column (100 × 2.1 mm; *i.d.* 1.8 µm) maintained at 25 °C, with aqueous 0.1% formic acid (*v/v*) as the mobile phase at a flow rate of 0.3 mL min^{-1} and isocratic elution. A sample aliquot of 2 µL was injected into the UPLC system and the L-AA peak was detected at 245 nm, identified and quantified by comparison with a commercial L-AA standard.[10-12] The limit of detection (LOD) and limit of quantification (LOQ) for the method were 1.0 and 3.0 mg L, respectively. An external calibration curve of L-AA was used for quantification, and vitamin C concentrations were expressed as mg 100 g DW.[10-12, 23]

Infrared spectroscopic measurements (MIR and NIR)

Dry powder samples were scanned using a Bruker Alpha fitted with an attenuated total reflectance a platinum diamond Attenuated total reflection single reflection module (Bruker Optics GmbH, Ettlingen, Germany). The MIR spectra were recorded using OPUS software, version 8.5, provided by Bruker Optics (Bruker Optics GmbH). Measurements were recorded in the spectral region between 4000 and 400 cm^{-1}. Each spectrum was computed using the average of 24 interferograms at a resolution of 4 cm^{-1}. Air was used for the reference background spectra. The ATR cell was cleaned with 70% ethanol and dried with paper wipes between samples to minimize the carry over between samples. The Fourier transform near-infrared (FTNIR) spectra of the dry powders was collected using a Bruker Tango (Bruker Optics GmbH, Ettlingen, Germany) with a gold-coated integrating sphere (diffuse reflection). Samples were placed in a glass cuvette with a 10 mm diameter (Bruker Optics GmbH). The NIR spectra was recorded using OPUS software, version 8.5, provided by Bruker Optics setting at 64 interferograms and a resolution of 4 cm^{-1} in the wavenumber range of 11 550 to 3950 cm^{-1}. The cuvettes were cleaned with 70% ethanol and dry with paper wipes between samples.

Calibration development

The spectra were exported from the OPUS software (OPUS format) to The Unscrambler X software version 11 (CAMO ASA, Oslo, Norway) for data processing and calibration development. Both the MIR and NIR spectra were pre-processed using the second derivative (Savitzky–Golay algorithm, second polynomial order, smoothing window size of 10 points).[24] The second derivative has been used in this study as it has been shown to be effective at correcting for baseline effects and slope of a spectrum.[24] Calibration models between the spectra (MIR and NIR) and UPLC-PDA reference data were developed using a partial least squares (PLS) regression. The optimal number of factors for the calibration model was selected based on the minimal value of the predicted residual sum of squares (PRESS) and the highest correlation coefficient (R^2) between actual and predicted values.[25] The PLS models were evaluated in terms of the number of factors, standard error of cross-validation (SECV), and correlation coefficient. The residual predictive value (RPD) was used to evaluate the accuracy of the models.[25]

RESULTS AND DISCUSSION

Table 1 shows the descriptive statistics (average, range, standard deviation) for the concentration of vitamin C measured in the KP powder samples and used to develop the calibration models. The vitamin C levels found in the fruit powder samples were in agreement with those reported previously by Williams and collaborators (2014) (14 038 mg 100 g DW) and Konczak and collaborators (15 190 mg 100 g DW).[8-9] Some samples also showed very high levels of vitamin C (>25 000 mg 100 g DW) corresponding with fresh, wild harvested KP fruit samples. These results indicated a wide range of variation in the data set due to the different

Table 1. Descriptive statistics for vitamin C determined by UPLC-PDA in Kakadu plum powder samples, calibration and validation sets

	Average	Standard deviation	Minimum	Maximum	CV%
Calibration (n = 80)					
Vitamin C (mg/100 g DW)	14 323.4	7433.6	227.4	28 954.3	51.9[a]
Validation (n = 5)					
Vitamin C (mg/100 g DW)	15 288	2973.6	13 261	20 390	19.4

a CV = coefficient of variation [(standard deviation/average) × 100].

sample sources (e.g. wild harvested, commercial samples). The variability in the data set was considered adequate to develop calibrations for this bioactive compound using vibrational spectroscopy.

Figure 1 (panel A) shows the average and standard deviation for the MIR and NIR spectra of the KP powder samples analyzed. The main bands observed in the MIR spectra are associated with moisture in the range between 3700 and 3100 cm^{-1} (O—H stretch); at 1640 cm^{-1} (O—H bend) overlap with protein bands and 1650 cm^{-1} (amide I), and 1450 cm^{-1} (amide II).[18] The O—H bending due to a small shoulder at 1650 cm^{-1} gradually disappeared as the concentration of vitamin C increased. The intensity or absorbance of C—C (1682 cm^{-1}) also increased with increasing vitamin C and overlaps with the band at 1650 cm^{-1}.[18] Two main bands were evident, one at 1100 cm^{-1} and the other at 1050 cm^{-1}, associated with structural and non-structural carbohydrates.[18] In this study, the fingerprint region was used to develop the PLS calibrations for vitamin C (1800 to 600 cm^{-1}). The NIR spectra of KP powder samples is showed in Fig. 1 (panel B). The main absorbance values were found around the O—H region around 6900 cm^{-1} (O—H stretch overtone), O—H combination tones (5417–4495 cm^{-1}) and around 4800–4200 cm^{-1}, which can be associated with N—H and C—H stretching modes (4397 cm^{-1}) and proteins (4812 cm^{-1}), respectively.[18, 26-27] Additional regions were often included in the models at higher wavelengths generally excluding the water overtones (7274–6338 cm^{-1} and 5417–4495 cm^{-1}).[18, 26-27]

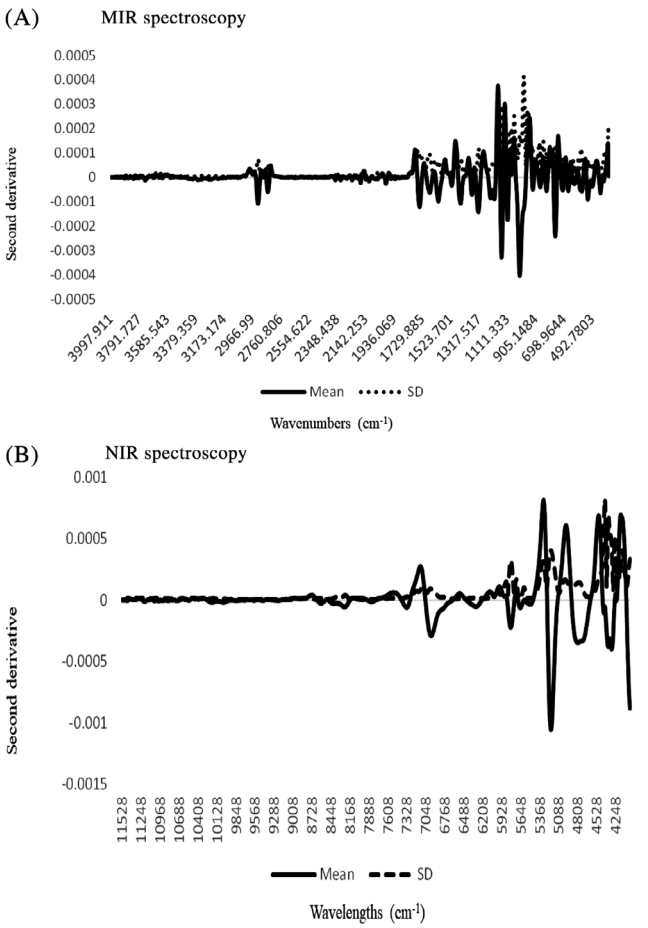

Figure 1. Average and standard deviation (SD) of the (a) mid and (b) near infrared spectra of Kakadu plum samples.

Table 2 shows the cross-validation statistics for the measurement of vitamin C using MIR and NIR spectroscopy. The coefficient of determination (R^2) and the standard error in cross validation (SECV) for vitamin C were 0.93 and 1811 mg 100 g DW and 0.91 and 1839 mg 100 g DW using MIR and NIR spectroscopy, respectively. The R^2 indicated that more than 90% of the variation was accounted for by the PLS regression models developed. The RPD values obtained for the calibrations were higher than 4 (RPD = 4.1 for MIR; RPD = 4 for NIR).[25] It has been reported that, in most of the infrared applications, RPD values higher than three are considered good for screening purposes, while RPD values higher than five are good for routine quality control.[25] The results of this study indicated that the PLS calibration models developed for vitamin C in the set of KP powder samples analyzed can be used to either screening samples for high, low, or medium content of vitamin C, or for routine quality control were high precision is not needed. The PLS calibration models indicated that vibrational spectroscopy (e.g. MIR and NIR) might substitute the use of UPLC-PDA during routine analysis or fruit screening at the point of harvest. The optimal number of latent variables used by the PLS models were four and eight for the models developed using MIR and NIR spectroscopy, respectively. The coefficient of correlation and the standard error of prediction (SEP) obtained using the independent set (n = 5) of KP powder samples were 0.65 (SEP: 2367 mg 100 g DW) and 0.73 (SEP: 4773 mg 100 g DW) using MIR and NIR, respectively. Figure 2 shows the scatter plots for the reference versus predicted values for vitamin C using MIR and NIR spectroscopy. It was observed during calibration development that the two samples having high concentrations of vitamin C were considered as outliers for the NIR models. The PLS loadings were also analyzed for the optimal models and they are reported in Table 3. The loadings for the MIR models were 1148, 1078, 987, 927, 849, and 760 cm^{-1}.[18, 26-27] The main loadings of the NIR models were observed around 6952, 5872, 5712, 5304, 4848, 4400, and 4250 cm^{-1} O—H and C—H.[18, 26-27] Vibrational spectroscopy methods (MIR and NIR) have been used

Table 2. Cross validation statistics for determination of vitamin C (mg/100 g DW) in Kakadu plum using mid and near infrared spectroscopy

	R^2	SECV	bias	slope	SEP	RPD	LV
MIR	0.93	1811	148.4	0.92	2367	4.1	4
NIR	0.91	1839	−0.69	0.91	4773	4.0	8

Abbreviations: LV, latent variables; MIR, mid infrared; NIR, near infrared; R, coefficient of determination; RPD, SD/SECV; SECV, standard error in cross validation; SEP, standard error of prediction.

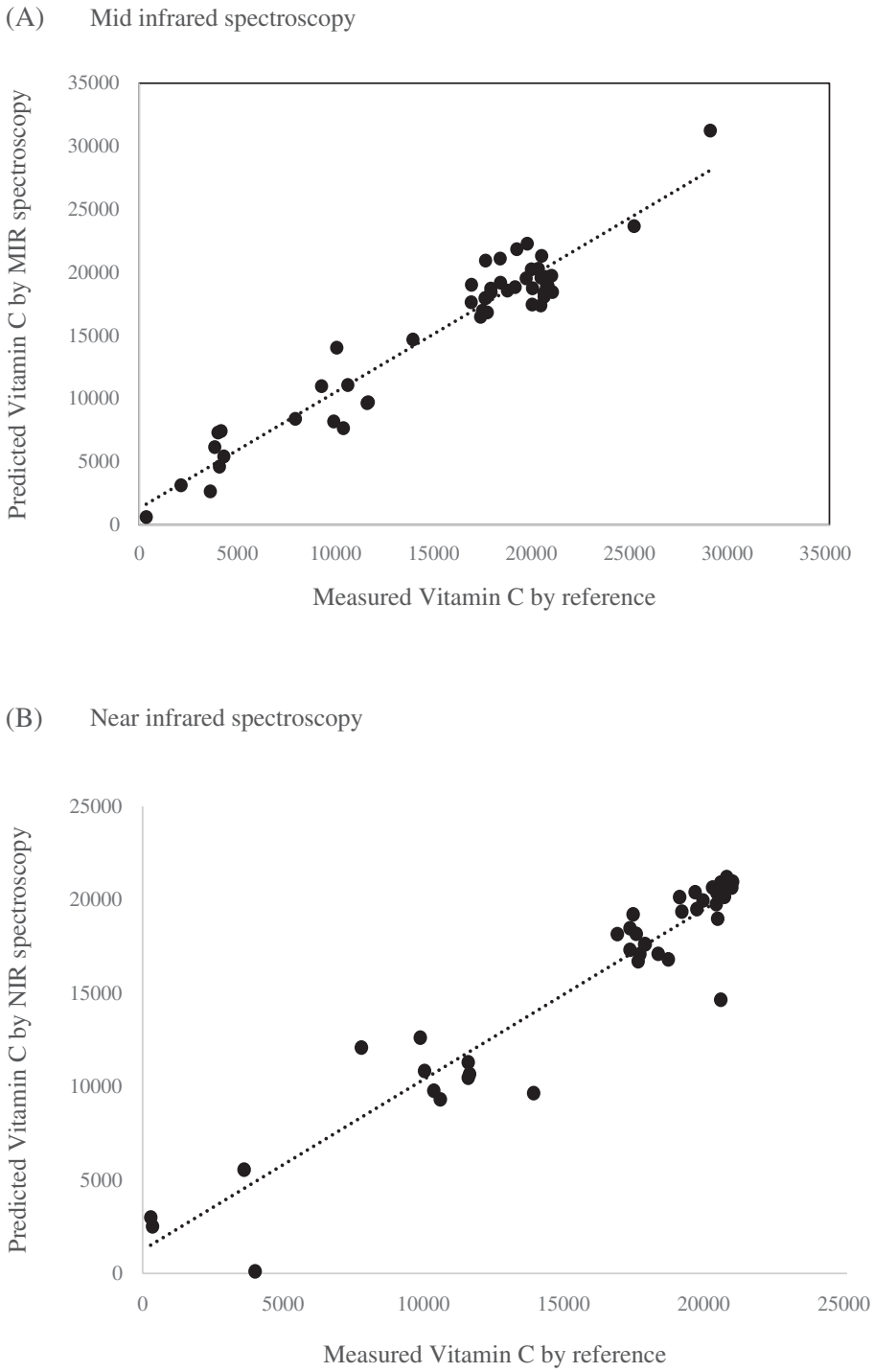

Figure 2. Reference *versus* predicted values for vitamin C (mg/100 g DW) using (a) mid and (b) near infrared spectroscopy.

Table 3. Partial least squares loadings derived from the determination of vitamin C using mid and near infrared spectroscopy

MIR (cm^{-1})	Assignation[18]	NIR (cm^{-1})	Assignation[26]
1148	C-O-C stretch and C-O-H bend	6952	C-H_2 combination
1078	C-O-C stretch and C-O-H bend	5872	C-H
987	C-H bend	5712	C-H
927	C-H bend	5304	O-H
849	C-H bend	4848	N-H
760	C-H bend	4400	O-H; C-O; C-H
569	C-H bend	4256	C-H

for the qualitative and quantitative characterization of food ingredients and other agricultural products. In particular, spectral analysis in the MIR region is the preferred technique to analyze natural ingredients. Compared with NIR, MIR spectroscopy is preferred for qualitative analysis because of its high signal-to-noise ratio, sensitivity, resolution, and narrower bands. However, in high moisture-content samples, a very strong absorbance in the MIR region might overlap with the important bands associated with the measured property. In this case, the use of NIR is preferred. Overall, the use of both MIR (ATR) and NIR spectroscopy provided with an alternative to analyze samples without the need of extensive sample pre-treatment or processing allowing for the development of a high-throughput analytical method.

CONCLUSIONS

The results demonstrated the great potential of either MIR or NIR to predict vitamin C in KP powder samples. The results obtained in this study show that it is possible to calibrate IR spectroscopic instruments for the measurement of vitamin C in KP powder samples. Mid-infrared spectroscopy showed the most promising results; however, FTNIR spectroscopy also rendered models capable of good quantification of this important bioactive compound in KP powder plants. Both instruments showed satisfactory robustness (e.g. SECV and SEP) to address the concerns of applying this technology in industry. However, one of the limitations of this study is the number of samples analyzed, and further validation must be recommended using samples from other sources, regions, and harvest seasons.

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AUTHOR'S CONTRIBUTIONS

A.P. and D.C. performed chemical analysis. A.P. and D.C. analyzed the data. Y.S., M.N., H.S., and D.C. designed experiments. All the co-authors contributed equally in drafting and revising the manuscript.

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